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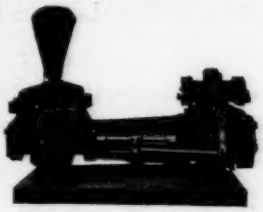

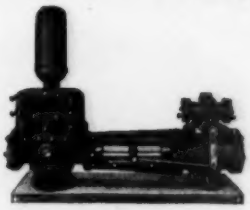

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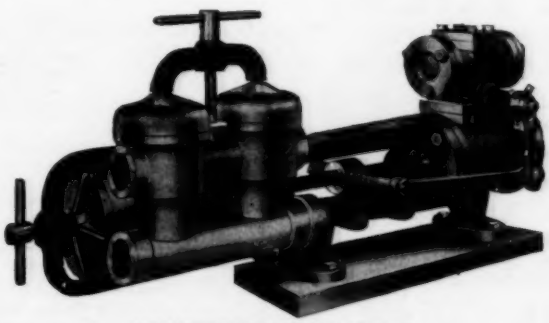

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THE CAUSES OF LEAKY BUTTER

A. C. DAHLBERG[1]

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INTRODUCTION

The moisture and salt content of most butter packed and stored in the usual way gradually decreases as the butter becomes older. The brine comes to the outside of the butter and either runs away or evaporates. This movement of brine from within the butter to the outside is commonly spoken of as leakage, and butter in which this movement is especially pronounced is called leaky butter. Leaky butter loses rapidly in weight due to the loss of brine, and this loss in weight is known as shrinkage. When leaky butter is cut into prints considerable brine is forced out which results in a marked decrease in weight.

Wet looking butter is butter that shows much free brine on its surface in the churn, and especially when a sample is taken with a butter trier after the butter has hardened in the refrigerator. Wet looking butter is commonly called leaky although no experimental work has been done to show that the two are the same.

A review of the dairy literature reveals the fact that no systematic attempt has ever been made to determine all of the conditions of manufacture that make butter leaky. Text books on butter making give a variety of reasons for the causes of leaky butter. McKay and Larsen, in their book, Principles and Practices of Buttermaking, state: ". . . . this leaky condition is brought about chiefly by churning the butter to small granules, washing the butter very little in cold water, salting heavily while butter granules are still small and firm, and working the butter frequently in the presence of brine." Martin H. Meyer in his

[1] This work was done by the author under the supervision of Prof. E. H. Farrington at the University of Wisconsin.

Modern Buttermaking discusses "leaky or slushy butter" as being caused by not cooling the cream sufficiently before churning, or by churning it too soon after it is cooled. It may also be caused by washing the butter with too warm or too cold water. Slushy butter is more apt to appear in the spring than at any other time of the year. The Book of Butter by E. S. Gutherie, states, "Often the butter is not worked sufficiently to incorporate the moisture properly, in which case the water collects in pockets. When this butter is cut, it appears leaky." O. F. Hunziker, in his book The Butter Industry discusses leakage of butter more extensively than the other writers. The conditions which he gives as the causes of leaky butter are too high a churning temperature, churning too quickly after cooling to churning temperature, insufficient working, rich cream, and tearing the butter apart rather than squeezing it together during working. This author also states: "Within relatively narrow limitations leakiness may be intensified or minimized by the processes of washing, salting, and working, but the fundamental cause of leakiness lies prior to these processes; it has to do with the treatment the cream receives preparatory to the churning process."

METHOD USED TO DETERMINE LEAKINESS OF BUTTER

The butter was packed out of the churn in so-called leakage tubes. The leakage tube consisted of a cylindrical tin can holding 28 pounds of butter which was wide open at one end and tapered like a funnel at the other. A 50-cc. burette was attached to the funnel end by means of a rubber stopper, and the lower end of the burette was plugged with a rubber stopper. The brine was caught in the burette and was measured as rapidly as it ran out of the butter. The upper part of the butter was cut level with the top of the tin can and was sealed by means of a large sheet of wrapping paper dipped in hot paraffin, which was placed on the butter before the paraffine hardened. The leakage tubes were, therefore, water-tight and the brine drained off the butter as rapidly as it leaked out. Readings in cubic centimeters of brine were taken for a period of seven days. Experiments proved that this was sufficient time in which to obtain repre-

sentative results. By the use of these leakage tubes a leakage of 1 cc. of brine or approximately 0.0025 of a pound could be determined, and this method was far more sensitive than the old method of weighing the butter to the tenth of a pound.

The effect of each variation in the manufacture of butter on its leakiness was compared with a normal or check churning from the same batch of cream churned in the same churn, a Perfection No. 6. Half of the cream in the vat was churned in the usual way, and two leakage tubes were packed. The remaining half was churned with the variation in handling the cream or butter which was being tried for its effect on leakiness. Two more leakage tubes were packed from this butter. The results of three sets of churnings, six churnings in all, from three different vats of cream and all determining the effect of one factor were considered sufficient evidence on which to draw conclusions.

EFFECT OF SIZE OF CHURNING ON LEAKINESS OF BUTTER

The cream in this experiment was divided into two unequal sized portions which were churned in the same identical manner. The larger churnings were normal sized and the smaller ones contained just enough butter to completely cover the shelf in the churn and be properly worked. Table 1, giving the weight of butter in each churning with the leakage expressed as cubic centimeters of brine and also as the shrinkage per hundredweight of butter, is a brief summary of the results obtained.

It is evident from table 1 that the butter from the small sized churnings was uniformly less leaky than butter from the larger churnings. The less leaky butter also appeared drier in the churn. The butter from vat no. 30 leaked twice as much as the butter from vat no. 28. No attempt was made to hold all conditions uniform from day to day, and a comparison of the butter made from the various vats of cream with each other is not justifiable. The butter from one churning can be compared only with its counterpart from the same vat of cream.

TABLE 1

Effect of size of churning on the leakiness of butter

VATS OF CREAM	WEIGHT OF BUTTER	SEVEN DAYS LEAKAGE	
		Brine	Shrinkage per hundred weight of butter
	pounds	*cc.*	*pounds*
No. 28	270	71.4	0.604
	460	106.5	0.900
No. 29	270	94.1	0.799
	498	140.8	1.193
No. 30	211	131.3	1.110
	348	187.8	1.586
Average	250	99.9	0.846
	435	145.0	1.228

TABLE 2

Effect of churning temperature on leakiness of butter

VATS OF CREAM	CHURNING TEMPERATURE	SEVEN DAYS LEAKAGE	
		Brine	Shrinkage per hundred weight of butter
	°F.	*cc.*	*pounds*
No. 12	55	32.5	0.275
	50	32.2	0.271
No. 13	54	4.5	0.038
	48	42.9	0.363
No. 14	54	25.0	0.211
	48	7.0	0.059
Average	54	20.7	0.175
	48	27.4	0.231

EFFECT OF CHURNING TEMPERATURE ON LEAKINESS OF BUTTER

One-half the cream was churned at the usual temperature and the other half was churned 6° too warm. The butter was washed at such a temperature that the finished butter would be of the

same temperature and hardness regardless of the temperature of the cream when it went into the churn. The results obtained are given in table 2. They show that although the butter churned warm did not look very much more wet in the churn than the butter from the colder churnings, the churning temperature had no effect on the rate of leakage. The churning temperature is a negligible factor in causing leaky butter provided the butter is washed at such a temperature that the butter has the proper firmness during working.

EFFECT OF TIME HELD COLD AFTER PASTEURIZATION ON LEAKINESS OF BUTTER

After pasteurizing the vat of cream one-half was churned as soon as the cream was at the churning temperature and the other half was held cold over night, or about twenty hours, before it was churned. The butter from the cream churned without having been held cold was very soft and warm so it was necessary to wash it much colder in order that the temperature of the finished butter would be the same as that of the butter churned from the cream which was held cold for twenty hours. The results set forth in table 3 show that although the butter made from the cream churned without holding cold after pasteurization looked very much more wet in the churn, the actual leakage was not any greater than that of the butter held cold for twenty hours after pasteurization.

EFFECT OF TEMPERATURE OF WASH WATER ON LEAKINESS OF BUTTER

The two churnings from the same vat of cream were handled in the same manner excepting that the wash water of one was 10°F. warmer than that of the other. This resulted in 5° difference in the temperature of the finished butter which was not enough to injure the body of the warmer butter. The data giving the results of this experiment are summarized in table 4. The butter washed warm was very soft when made; it appeared a little more wet in the churn, but held its moisture on standing

much better than the butter washed cold. This result is directly opposed to present opinion, but these results and others obtained on the shrinkage of butter in cold storage prove conclusively

TABLE 3

Effect of time held cold after pasteurization on leakiness of butter

VATS OF CREAM	HELD COLD	SEVEN DAYS LEAKAGE	
		Brine	Shrinkage per hundred weight of butter
	hours	*cc.*	*pounds*
No. 31	0	141.1	1.193
	20	128.6	1.088
No. 32	0	105.0	0.888
	20	100.5	0.850
No. 33	0	8.9	0.075
	20	3.9	0.033
Average	0	84.7	0.716
	20	77.7	0.657

TABLE 4

Effect of temperature of wash water on leakiness of butter

VATS OF CREAM	TEMPERATURE OF WASH WATER	SEVEN DAYS LEAKAGE	
		Brine	Shrinkage per hundred weight of butter
	°F.	*cc.*	*pounds*
No. 9	54	137.2	1.161
	65	28.9	0.245
No. 10	55	165.8	1.403
	65	34.8	0.293
No. 11	55	119.2	1.008
	65	26.5	0.224
Average	55	144.9	1.226
	65	30.0	0.254

that butter which is soft when worked holds its moisture much better than hard butter, providing both are worked the same number of revolutions.

In the experiments to determine the effect of the time the cream was held cold after pasteurization before churning, and the effect of the churning temperature on leakiness, the temperature of the wash water was such that the finished butter always was the same hardness. If the wash water had been used at the same temperature regardless of the condition of the butter, then a warm churning temperature and churning immediately after pasteurization would have resulted in a soft butter that would have been less leaky. If no variation is made in the amount the butter is worked the common opinion that churning immediately after pasteurization, churning at a warm temperature, and washing butter warm causes butter to be leaky because it is wet looking is incorrect.

EFFECT OF THE AMOUNT OF WATER IN THE CHURN DURING WORKING OF THE BUTTER ON ITS LEAKINESS

The leakiness of butter worked in a dry churn was compared with butter worked in brine. The churn was made dry by placing a short piece of cork in each door so that the doors when closed remained about $\frac{1}{4}$ inch open. Whenever the churn contained water it was stopped with the doors on the under side and all water was drained out. To the butter worked in water $2\frac{1}{2}$ pails of strong salt brine of the same temperature as the butter were poured into the churn and the doors were tightly closed. The butter worked in water contained more moisture, looked more wet in the churn, and the results stated in table 5 show that it was more leaky than butter worked in a dry churn. This confirms the practice of many creameries of finishing the working of the butter in a dry churn for the purpose of making the butter non-leaky.

EFFECT OF AMOUNT BUTTER IS WORKED ON ITS LEAKINESS

When the butter had been worked just enough to remove all grittiness and prevent mottles, two leakage tubes were filled. The remaining butter was worked until it appeared close grained and waxy, and two more leakage tubes were filled. This experi-

ment should not be interpreted as indicating the correct number of revolutions that butter should be worked, for commercial practice has demonstrated that the amount of working butter

TABLE 5

Effect of amount of water in the churn during working of the butter on its leakiness

VATS OF CREAM	BRINE IN CHURN	SEVEN DAYS LEAKAGE	
		Brine	Leakage per hundred weight of butter
	gallons	*cc.*	*pounds*
No. 23	0	3.8	0.032
	7	104.0	0.980
No. 24	0	40.4	0.342
	7	53.3	0.451
No. 25	0	87.7	0.666
	7	175.5	1.485
Average	0	40.9	0.346
	7	110.9	0.938

TABLE 6

Effect of amount butter is worked on its leakiness

VATS OF CREAM	REVOLUTIONS WORKED	SEVEN DAYS LEAKAGE	
		Brine	Leakage per hundred weight of butter
		cc.	*pounds*
No. 6	20	98.6	0.834
	40	0.1	0.001
No. 7	20	61.2	0.518
	30	0.7	0.006
No. 8	20	180.1	1.524
	30	1.1	0.009
Average	20	113.3	0.958
	33	0.6	0.005

should receive is variable. The amount this experimental butter was worked represents the minimum rather than the maximum working that butter should receive.

The data presented in table 6 give very contrasting leakages. The butter worked the greater number of revolutions looked very dry in the churn and held its moisture exceedingly well. As a result of observations on all the churnings reported in this paper it became very evident to the author that the amount butter is worked has a greater effect of leakiness than any other individual factor, and butter which might otherwise be leaky could be made non-leaky by working it as much as possible without injury to the body of the butter.

All of the butter made in this experiment was scored by competent butter judges and no criticisms were made of the body, color or salt. One must conclude that either (1) the butter judges were very lenient in considering the body of the butter because the butter which was made from vat no. 8 and worked 20 revolutions lost 1.5 pounds in seven days, while the butter from the same vat of cream that was worked 30 revolutions lost only 0.009 pounds in seven days, or (2) the butter judges could not distinguish between leaky and non-leaky butter.

EFFECT OF STORAGE TEMPERATURE ON LEAKINESS OF BUTTER

Four leakage tubes were filled from the same churning, two of which were held in a warm room and two in the refrigerator. The data contained in table 7 gives conclusive evidence that butter held at a cold temperature leaks brine more rapidly than if held at a warm temperature. This fact was very noticeable throughout the entire work, and it is one of the reasons for an uneven leakage from day to day. Whenever the refrigerator became very cold the leakage was always greater, and the leakage could be almost stopped by a warm storage temperature. It must be remembered, however, that the temperatures employed were not cold storage temperatures, and the results obtained do not necessarily apply to freezing temperatures. If this experiment had been so conducted that evaporation took place from the butter it is very probable that different results might have been obtained.

COMPARISON OF LEAKINESS OF BUTTER PRODUCED IN SUMMER AND WINTER

Churnings of butter produced under winter conditions were made to compare with churnings of butter produced under summer conditions. The winter butter was produced in the early part of April and the cattle were fed hay, grain, and silage. The summer butter was produced in June and the cattle received little or no supplement to the pasture. In every churning the finished was the same number of degrees above the churning temperature and as firm in the summer as in the winter.

TABLE 7

Effect of storage temperature on leakiness of butter

VATS OF CREAM	TEMPERATURE OF STORAGE	SEVEN DAYS LEAKAGE	
		Brine	Leakage per hundred weight of butter
	°F.	cc.	pounds
No. 20	45	12.7	0.107
	63	0.6	0.005
No. 21	48	35.0	0.296
	64	4.1	0.035
No. 22	48	89.0	0.753
	65	3.0	0.025
Average	47	45.6	0.387
	64	2.6	0.022

The results of these comparisons gave a slightly greater total leakage for the summer butter. It was difficult to duplicate conditions exactly with a spread of two months between churnings and individual variations between churnings were so great that the differences obtained in the total leakage became insignificant. It must be concluded therefore that little or no difference exists between the leakage of summer and winter butter when only the composition of the butter is concerned. The results of other experiments reported in this paper indicate that the heavy shrinkage in butter often noted in commercial work

FIG. 1. LEAKY AND NON-LEAKY BUTTER PACKED INTO ASH TUBS

Note the water logged and stained condition of the tubs at the left containing leaky butter as contrasted with the drier, whiter tubs at the right containing non-leaky butter. This difference in the appearance of the tubs is usually no index of the leakiness of the butter they contain because tubs vary so greatly in their absorptive powers.

during the spring months is probably caused by the use of cold wash water and a small amount of working usually employed to prevent a salvy, greasy body and to lower the moisture content of the finished butter.

EFFECT OF SALT CONTENT OF BUTTER ON ITS LEAKINESS

An excessive amount of salt was added to the first churning and an average amount was added to the second churning from the same batch of cream. None of the butter was gritty. The data summarized in table 8 show that the highly salted butter leaked brine about twice as rapidly as the butter which contained a smaller amount of salt.

TABLE 8

Effect of salt content of butter on its leakiness

VATS OF CREAM	SALT	SEVEN DAYS LEAKAGE	
		Brine	Leakage per hundred weight of butter
	per cent	*cc.*	*pounds*
No. 15	3.8	283.0	2.394
	2.2	166.0	1.404
No. 16	5.0	325.0	2.749
	2.7	170.0	1.442
No. 19	3.7	228.3	1.931
	2.1	46.6	0.392
Average	4.1	269.5	2.280
	2.3	127.5	1.079

EFFECT OF MOISTURE CONTENT OF BUTTER ON ITS LEAKINESS

It was practically impossible to make two churnings from the same vat of cream in such a way that there would be a marked difference in the moisture content of the finished butter without introducing some variation in the method of manufacture which would also affect the leakiness of the butter. Consequently, to determine the effect of the moisture content of butter on its leakiness all the churnings previously made were divided into

two groups. The leaky butter group includes those which were the more leaky of the two churnings from the same cream. The non-leaky butter, was that which leaked the least. All butter which showed no difference in the leakage from duplicate churnings were not included in the data. Table 9 summarizing the data is based on an average of thirty churnings.

The moisture content of the butter did not vary according to the leakiness. One sample containing 13.2 per cent of water was leaky, another containing 15.8 per cent was dry, and the average moisture content of the two lots of butter was quite uniform.

TABLE 9

Relation of moisture content of butter to its leakiness

	AVERAGE MOISTURE CONTENT	HIGHEST MOISTURE CONTENT	LOWEST MOISTURE CONTENT
Leaky butter	14.8	16.2	13.2
Non-leaky butter	14.3	15.8	13.3

IS WET LOOKING BUTTER LEAKY?

That butter which looks wet does not necessarily contain a high percentage of moisture has been shown in the past and the work was again repeated. Butter entered in a Wisconsin scoring exhibition was classified according to the free moisture noticed when samples were taken with a trier and it was then tested for moisture and salt. Table 10 summarizes the results.

No relationship exists between the percentage of water in butter and its appearance in respect to visible moisture. However, dairymen have always assumed that butter which looks very wet is also very leaky. The two terms are used interchangeably. The author previously thought this belief was correct and unfortunately did not in the beginning of this experimental work attempt to determine if butter which was leaky actually showed a wet trier sample. The methods of manufacture which causes butter to look wet have been observed in practice and table 11 compares these causes with the causes of leaky butter found as a result of this experimental work.

Factors which cause butter to look wet do not necessarily cause it to be leaky. Therefore wet looking butter is not always leaky, although they are doubtless closely associated with each other.

Butter looks wet because some of the moisture in it exists in large droplets and in pockets or holes in the butter. On a

TABLE 10

Relation of moisture content of butter to its apparent wetness

HOW THE BUTTER LOOKED	NUMBER OF TUBS	WATER	SALT
		per cent	*per cent*
Very dry	13	14.4	2.0
Dry	56	14.1	2.1
Little wet	51	14.2	2.1
Wet	12	14.6	2.2
Very wet	9	13.2	2.2
Average	141	14.1	2.1

TABLE 11

Comparison of the causes of wet looking and leaky butter

FACTOR TRIED	EFFECT ON LEAKAGE	EFFECT ON APPEARANCE
Large churning	Little leaky	No effect
Warm churning temperature	No effect	Wet
Not held cold after pasteurization	No effect	Wet
Warm wash water	Dry	Wet
Working butter in water	Leaky	Wet
Under working butter	Leaky	Wet
Warm refrigerator	Dry	Wet
High salt content	Leaky	Little wet
Butter high in soft fat	No effect	Wet
High moisture content	No effect	No effect

freshly cut surface this water can readily be seen and it is forced out by a trier when taking a sample. Butter is leaky because it has an open texture, or, in other words, is full of small capillarities through which the water can pass. The moisture which leaks out of butter does not simply run out like water running in a stream, but the moisture moves by capillarity. This is shown by the fact that after heavy leakage the butter always contained a

uniform moisture content throughout the entire mass, the percentage of water being the same at the top, bottom, sides and middle of the butter. Conditions in manufacture which made the butter-fat globules hard and preserve them in their natural spherical shape tends to keep the spaces between the globules as large as possible. Washing butter cold and underworking give such results. If butter is stored in a cold refrigerator the butter fat globules contract and the spaces between them must become larger. Hence, the butter becomes more leaky. Although some factors, such as a high salt content, which cannot affect the size of the capillarities did materially increase leakage, their effect can be almost entirely eliminated by reducing the size of the capillarities to such an extent that all leakage practically ceases. Their effect must, therefore, be considered of secondary importance.

PRACTICAL APPLICATION OF RESULTS OBTAINED

During the summer months butter was carefully made for cold storage with variations in the method so that the leakage would be affected. The same vat of cream was often divided and churned to make one-half of the butter leaky and the other half non-leaky. The butter was packed into paraffined 63-pound ash tubs. It was weighed net to the tenth of a pound as it came from the churn, and was re-weighed in like manner after four to seven months in cold storage. Table 12 summarizes the results. The results obtained with cold storage butter agree with those obtained by the leakage tubes. It is especially noteworthy that as small a difference as ten revolutions in the amount the butter was worked caused a variation of 100 per cent in the rate of skrinkage or leakage. The butter washed with very cold water leaked about five times as much brine as the butter washed with very warm water and the rate of leakage of butter washed at an average temperature was between these two extremes. The moisture content of some of the leaky butter decreased as much as 4 per cent while most of the drier butter lost about 1 per cent moisture.

It has been shown that various conditions affect the leakiness of butter. Some conditions which make butter retain its moisture tend to produce butter of poor quality and it is not advisable to injure the butter in any way to make it less leaky. Other conditions which make butter non-leaky cannot be practically used. The working of butter influences the leakiness to the greatest extent and should be used as the means of controlling shrinkage. Wash butter with a cold water so that the body will be medium firm, even though this caused it to be more leaky. Such butter needs much extra working to give it the proper waxy body, and when it has been worked sufficiently it will be of good body, close grained, dry looking and will retain its moisture

TABLE 12

Variations on shrinkage of cold storage butter

VARIATION IN MANUFACTURING	NET WEIGHT BEFORE STORAGE	NET WEIGHT AFTER STORAGE	LOSS	
			pounds	*per cent*
Average of 234 tubs	14,655.4	14,435.5	219.9	1.50
Normal temperature of wash water	9,577.4	9,447.8	129.6	1.35
Very cold wash water	831.4	795.9	35.5	4.27
Very warm wash water	1,425.1	1,414.5	11.2	0.78
Worked normal number of revolutions	1,440.5	1,411.5	29.0	2.01
Worked ten more than normal	1,380.4	1,365.8	14.6	1.05

better than if made in any other way. The extra working causes it to hold its moisture and is permitted without injury to the body of the butter, by the use of cold temperatures.

In judging the leakiness of butter by a trier sample, two conditions should be noted: first, the openness of texture, and second, the amount of free brine. Close grained butter is not leaky even though it may appear wet. An open texture and much free brine indicate a leaky butter, but the amount of free brine alone is not a satisfactory index of the leakiness of butter.

CONCLUSIONS

Some of the results obtained from the experimental work reported in this article are contradictory to text book teachings

and the opinions of many practical creamery men. For that reason the conclusions drawn from the data presented are enumerated and briefly summarized as follows:

1. The leakiness of butter is not affected by (1) the churning temperature, (2) time held cold after pasteurization previous to churning, (3) the moisture content of the finished butter, (4) high percentage of the soft butter fats.

2. Butter is made leaky by (1) large churnings, (2) cold wash water, (3) working butter in water, (4) not enough working after salting, (5) high salt content, (6) cold refrigerator.

3. The amount butter is worked has a greater influence on leakiness than any other factor and should be used to control leakiness.

4. Wet looking butter is not always leaky.

5. The openness of texture is the best indicator of the leakiness of butter.

6. Butter made leaky lost 4.2 per cent in weight during a period of six months in cold storage and butter made non-leaky lost only 1 per cent in the same length of time.

THE INFLUENCE OF CERTAIN FACTORS ON THE METHYLENE BLUE REDUCTION TEST FOR DETERMINING THE NUMBER OF BACTERIA IN MILK[1]

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One of the factors, and possibly the most important in determining the quality of milk is its bacterial content, hence, wherever a classification of milk is desired, a bacterial examination is essential. In the dairy manufacturing field, the payment for milk according to its bacterial content has made little headway. The future must bring this change into the industry if the quality of the products is to be maintained at even its present low level, to say nothing of the improvement that should take place. There is no reason why the same price should be paid for high quality milk, as for a milk that is sure to lower the grade of the product for which it is to be used. In the butter industry and in the market milk business certain curative measures can be employed to overcome the negligence of the producer in the production and handling of milk and cream. In the cheese industry, however, prevention, not cure, must be used. A difference in renumeration to the producer is the most powerful lever that can be employed to raise the quality of the milk no matter for what purpose it is intended.

Milk low in bacteria is, generally speaking, of higher quality for cheese making than is milk containing many bacteria. If the cheesemaker seeks to differentiate the milk of his patrons into grades, he must have some means of measuring the bacterial content. Again in the control of market milk the smaller cities have not kept up with the larger in the improvement of their

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

milk supplies. They have not been financially able to duplicate the methods of the larger cities. No simple and inexpensive method of determining the bacterial content of milk has been available until recently, and for this reason progress under many conditions has been difficult.

The methylene blue reduction test is the only one that seems to meet the necessary requirements, in so far as simplicity and expense are concerned, for use in many fields, especially in the field of dairy manufacturing. This test is based on the fact that the color imparted to milk by the addition of the dye, methylene blue, disappears more or less quickly, the time required for its disappearance depending on a number of factors, most important of which are the bacterial content of the milk and the temperature at which the test is made. If all controlling factors can be kept constant other than the bacterial content of the milk, the time required for the color to disappear will be determined by the number of bacteria.

The great service which such an inexpensive and simple test might yield to the dairy industries of this country has led the authors to review the literature and to study the test from many points of view. This paper presents simply a summary of our work. In connection with nearly every point considered, many tests have been made. Our conclusions are, therefore, based on much more numerous data than it is possible to present here. It is hoped that this paper may serve to bring the reduction test to the attention of many who may find use for it.

In spite of the widespread use of the reduction test in Scandinavian countries and in other parts of Europe, the method has not been adopted by the dairy industry of this country. Apparently the chief reason for its non-adoption is the belief that it is at best a rough method. The main basis for this conclusion is the fact that the results obtained with it do not correlate exactly with those supplied by the plate culture method. Rahn (1920) has recently emphasized this point and has concluded that the method is not an accurate one. Some of the reasons for the non-correlation of the two methods were given by Hastings (1919). Others will be presented in this paper.

EXPERIMENTAL WORK

The effect of the concentration of methylene blue on the reduction time

In the practical application of the methylene blue reduction test varying concentrations of the dye have been used. Fred (1911) used 1 part of the crystalline dye to 20,000 parts of milk, Jone (1915) 1 part to 50,000; Mueller (1906) 1 part to 40,000; and Kufferath (1919) 1 part to 3000-4000. The latter noted that sterilized milks, by which expression was meant milks heated to the boiling point but not actually sterile, did not reduce the dye. He also noted that raw milk very low in bacteria did not

TABLE 1

Effect of concentration of methylene blue on reduction time

RATIO OF CRYSTALLINE DYE TO MILK	MILK I	MILK II	MILK III	MILK IV
	minutes	minutes	minutes	minutes
1:10,000	25	60	165	
1:20,000	25	45	165	265
1:40,000	22	42	117	240
1:100,000	15	32	95	189
1:200,000	10	27	93	163
1:300,000	8	21	77	163
1:400,000	6		57	160

reduce the dye for several days. Simmons (1919) using a concentration of 1 to 10,000 noted that some milks did not reduce the dye.

It is a well known fact that methylene blue has an antiseptic action. It seems probable that some of the workers who have used the reduction test employed such amounts of the dye that it must have retarded to a marked extent the growth of the bacteria and therefore the reduction time was prolonged.

A considerable number of trials have been made to determine the effect of the dye on the reduction time and the concentration best adapted for employment in the test. Sufficient of the data obtained to illustrate the effect of the varying concentrations on the reduction time of different samples of milk are given in

table 1. It is to be noted from the data that the reduction time is prolonged as the concentration of the dye increases, a fact that can be explained only by the injurious effect of the dye on the activities of the organism.

The tablets prepared and sold by Blauenfeldt and Tvede of Copenhagen are commonly employed in making the reduction test in the Scandinavian countries where it is most widely used. When these tablets are used according to the directions furnished therewith, the color imparted to the milk is practically identical with that obtained when 1 part of the crystalline dye is used to 200,000 parts of milk. Tests made on 24 samples of market milk show that the reduction time is practically the same with the tablets as with 1 part of the crystalline dye to 200,000 parts of milk.

Influence of different brands of dyes

Dyes from various sources, both foreign and domestic, have been compared in different concentrations. The differences in reduction time with any sample of milk, when different dyes are used, are so small as to have no practical significance.

Several writers have stressed the importance of using dyes free from impurities. It seems probable that the impurities which may be present in the grades of dyes that should be used will have little if any effect on the results in the high dilutions in which the dye is employed.

Effect of the age of the methylene blue solution

A number of writers have made the statement that the methylene blue solution to be used in the reduction test should be freshly prepared. Others emphasize the necessity of using sterile solutions of the dye. In order to determine the effect of age on solutions of the dye, tests were made with freshly prepared solutions of dyes from various sources, and with solutions which were two years old. Some of the results are presented in table 2. The results obtained with the fresh solutions and with the old

solutions are practically identical. It is, therefore, believed that the age of the solution will have little if any effect in the practical application of the test.

TABLE 2

The comparative reduction time with fresh and old solutions of methylene blue

	MARTINDALE		HARMER	
	Fresh	Two years old	Fresh	Two years old
	minutes	minutes	minutes	minutes
1:100,000	425	400	146	148
1:200,000	400	370	139	137
1:300,000	380	360	135	137
1:400,000	353	350	121	113

The effect of temperature on reduction time

Most investigators have employed 37° to 38°C. in the reduction test. It is known that some of the milk bacteria, such as the lactic,[2] may grow more rapidly at slightly lower temperatures. It would, therefore, seem that a lower temperature might be more desirable in the practical application of the test. The reducing properties of various pure cultures at different temperatures have been studied by Rahn (1920) and the effect of varying temperatures on the reducing properties of market milk has been studied by Arup (1918) who believes that if milk has been stored at low temperatures, the reduction time at 30°C. will be shorter than at 38°C.

In order to gain some information concerning the reducing power of market milk at different temperatures, a number of tests were made. In each trial approximately 20 samples were employed. If the reduction time of a single sample or the average reduction time of a group of samples at 38°C. is expressed by unity, the reduction time at any other temperature may be expressed by some fraction or multiple of unity. With the great majority of samples the higher temperature has given the most rapid

[2] In this paper the phrase "lactic bacteria" is used to refer to that group of organisms which is primarily concerned in the souring of milk at ordinary temperatures.

reduction. With certain samples of milk the reduction time at 28°C. has been shorter than at 38°C. There seems, however, no reason to deviate from the accustomed temperature (38°C.) in the making of reduction tests. The relative rank of a series of samples is probably of greater importance than the actual reduction time, since it is desirable that the relative quality of milk from a number of sources be determined and improvement of the supply coming from those furnishing the poorer milks be sought. In general, a series of samples of milk will be placed in the same order by the reduction test no matter what the temperature employed in the making of the test. The temperature at which the average milk will reduce most quickly is, therefore, the best one to be used.

TABLE 3

The influence of temperature on the relative average reducing power of a series of samples of milk

TRIAL	38°C.	33°C.	28°C.
1	1	1.4	2.0
2	1	1.5	1.9
3	1	1.7	2.1
4	1	1.5	2.0
5	1	1.6	1.9
6	1	1.4	2.0

Are there non-reducing bacteria?

Previous investigations claim to have found that certain bacteria would not reduce methylene blue in milk. It seems to the writers that these observations are in error. An organism which grows slowly in milk, either because of temperature, nutritive conditions, or an excessive amount of methylene blue will not be able to use oxygen as fast as it can diffuse into the milk. Such an organism would be classed as a non-reducer. Under appropriate conditions such an organism will reduce the dye. In a mixture of organisms it will exert an effect. There is every reason to believe that every organism growing in the milk assists in the reduction of the dye. All do not function in the same degree, however.

The relation of the living cell to reduction

The effect of heat on the reduction time is evidence that the cell itself and not its enzymes are the causal agents in the reduction of the dye. The data presented in table 4 show that temperatures that are not supposed to affect enzymes influence the reducing action of milk. The increase in reduction time with the increase in temperature may indicate the varying resistance of different cells of the same organism or a complexity of bacterial flora in the milk, or possibly it may indicate both conditions. The

TABLE 4

The effect of heating milk to varying temperatures on its reducing power

TEMPERATURE TO WHICH HEATED	TRIAL 1	TRIAL 2
°C.	minutes	minutes
40	17	45
45	17	50
47	17	50
50	17	55
52	32	77
54	42	107
56	57	135
58	139	155
60	189	205
62	242	240
64	377	305

effect of such a weak antiseptic as boric acid in concentration of 1 part per 2500 parts of milk approximately doubles the reduction time. The effect of increasing amounts of methylene blue in lengthening the reduction time is to be traced undoubtedly to the antiseptic action of the dye. The influence of these factors, heat and antiseptics, finds its explanation in their influence on the living cells, and it is evidence that the reduction of methylene blue is very intimately connected with the vital processes of the cell rather than with any extracellular by-products.

Effect of reaction of the milk on reduction time

Mueller (1906) studied the effect of varying reactions on the reduction time and also studied the effect of adding sodium carbonate and sodium bicarbonate to sour milk. His conclusion from rather extended observations was that the reduction time of milk in which a considerable amount of acid has been developed is not changed by neutralization of the acidity. Orla-Jensen also made similar observations. This is exactly what one would expect for it is well known that the rapidity of growth of bacteria in milk does not decrease until the acidity has reached a point at which the milk will curdle at ordinary temperatures. In other words, the reaction of any sample of milk to which the reduction test would ever be applied in practice would not be such as to interfere with the test.

Effect of shaking on the reduction time

It is recognized that one of the reasons why the plate culture does not reveal the exact number of living cells in the milk is the occurrence of clumps of organisms therein. The ordinary lactic bacteria tend to occur in twos, other forms may occur in much larger aggregates. Each aggregate, of course, gives rise to a single colony. This has led the committee of the American Public Health Association that formulated standard methods for the bacteriological examination of milk to suggest that the plate culture count of milk be spoken of as such rather than to speak of the number of bacteria per cubic centimeter. This source of error in the plate culture method should not be present in the reduction test since undoubtedly each cell of such clumps as are likely to occur in milk will function as though the other cells were not present. This should indicate that shaking would have no effect upon the reduction time. Dons (1914) found this to be true. Orla-Jensen confirmed this observation. In our own work some attention has been paid to this point. We have found that the agitation of the milk has practically no effect on its reduction time.

Relationships existing between different groups of bacteria in milk

Efforts have been made by numerous investigators to correlate the results of the plate culture method with those obtained with the methylene blue reduction test. When the correlation was not an exact one, it has had the tendency to minimize the value of the reduction test. Rahn (1920), in a recent article, has again raised this objection and considers the reduction test only an approximate method and not comparable in value to other tests.

It seems apparent that these investigators have not given adequate consideration to the relationships which must exist between the various groups of bacteria that are present in milk. Two kinds of organisms may have a favorable reaction upon each other; thus the growth of both may be stimulated. Again, one kind may be favored by the previous growth of another, or the development of one group may exert an inhibiting action upon another. An important example of this is the influence of the acid-forming group upon the liquefying bacteria. Again, inhibition of both organisms may result. When one considers that there are many groups of organisms present in milk, it is evident that the relationships existing between them must be most complex. In the plate method these relationships are avoided when the plates are not too thickly seeded. It is well known to every bacteriologist that thickly seeded plates give no correct idea of the quantitative or qualitative bacterial content of the milk. Plates heavily seeded may give the impression that nothing but lactic bacteria are present in the milk, while more thinly seeded plates from the same sample may reveal many other kinds of bacteria. The existence of these relationships between different groups of bacteria in milk and their absence in plate cultures is undoubtedly an important cause for a lack of correlation between the results obtained with the plate culture method and with the reduction test.

From many points of view the bacteriologist is chiefly interested in those organisms which are actually growing in milk. It seems probable that the methylene blue reduction test because it is influenced by these relationships measures more accurately than does any other method the bacterial activity in the milk.

Variation in reducing action of different kinds of bacteria

Another cause for the non-correlation of results obtained by the plate culture method and by the reduction test is the variation in the reducing action of different milk bacteria. Efforts have been made by a number of investigators to compare organisms with reference to their reducing power. Orla-Jensen (1912), Barthel (1908), Weigmann and Wolff (1916) and Rahn (1920) have worked along this line. The conclusions which they reached in regard to the reducing action of the different milk bacteria were quite dissimilar. Orla-Jensen and Barthel asserted that the ordinary lactic bacteria were not to be classed as strong reducing organisms, while Rahn believed them to be the most active in the reduction test. The methods used by these investigators varied. Orla-Jensen and Barthel added methylene blue to the cultures that were already fully developed, while Rahn inoculated the milk with the culture and then added the methylene blue. In the latter instance the conditions would be exactly similar to those present in the practical application of the test; namely, the organisms would be actively growing during the time of the test while, in the method used by Orla-Jensen and Barthel, only the dormant organisms would be present.

In our own work many efforts have been made to obtain some idea of the relative reducing action of different bacteria. Various methods have been employed. The one which gave the most satisfactory results was as follows: A series of tubes of fresh milk which had been heated to the boiling point were inoculated with a constantly decreasing amount of an active lactic culture. The same tubes were then treated in the reverse order with the culture of the organism to be compared with the lactic. Each tube of the series, therefore, contained the same volume of the mixed cultures of the two organisms, but the relation of the two cultures to each other was different in each tube. If the organism to be compared with the lactic had the same reducing action, and if the organisms did not influence each other unfavorably and grew at approximately the same rate, the time for reduction should be much the same in the different tubes of the series. This con-

clusion is based on the supposition that the cultures of the various organisms contained approximately the same number of cells. Although such a condition probably did not exist nevertheless the variation in number should not influence the results to such an extent as to make them of no value. Some of the results obtained are given in table 5.

It is to be noted that with each organism tested the reduction time has increased as the inoculum with lactic decreased. If the compared organism had reducing powers equal to the lactic

TABLE 5

The reducing action of different bacteria in milk

INOCULUM			COMPARED ORGANISMS												
Tube	Lactic bacteria	Compared organism	1	2	3	4	5	6	7	8	9	10	11	12	13
	cc.	cc.	min.	min.	min.	min.	min.	min.	min.	min.	m'n.	min.	min.	min.	min.
1	1.9	0.0	8	10	14	12	13	12	31	15	15	15	15	8	8
2	1.7	0.2	11	12	16	14	15	13	37	15	15	15	15	11	8
3	1.4	0.5	16	22	20	18	15	19	43	20	20	16	20	16	16
4	1.1	0.8	21	26	30	20	19	25	64	24	24	24	24	22	22
5	0.8	1.1	29	35	30	28	30	38	71	33	33	33	33	35	35
6	0.5	1.4	43	62	56	50	45	58	81	44	45	45	80	70	70
7	0.2	1.7	80	87	71	57	52	106	98	67	56	56	67	120	120
8	0.0	1.9	140	173	93	95	85	178	335		107	260	260	360	185

Organisms 1 to 6 inclusive were isolated from milk. They represented the udder flora. Organisms 7, 8, and 9 were representatives of the aerogenes group; 10 and 11 of the colon group; 12 and 13 of the Bulgaricus group.

bacteria this increase in reduction time would not have occurred for the increasing amount of the compared organism would have balanced the decrease in lactics. No organism tested was at all comparable to the lactic in reducing power. The great difference in reduction time between tubes 7 and 8 is evidence of the marked reducing action of the lactic bacteria in comparison with the other bacteria. Tube 7 contained in each trial a small amount of lactic bacteria, tube 8 none.

The reducing action is, of course, influenced by the rate of growth of the various organisms. The temperature used in the

test, namely, 38°C., is more favorable for the organisms of the colon-aerogenes group than for those of lactic group. In spite of this handicap the lactic organism exhibits much greater reducing action than do the members of the colon group. This observation agrees with what is known concerning the reducing action of these organisms in litmus milk.

The relation of reduction time to keeping quality

The desirability of a laboratory test that would give some evidence of the keeping quality of milk would be of value in grading it. In order to collect some information as to keeping quality as measured by the development of acid, and the reduction time of the milk, a number of tests were made. In the first trial tubes of fresh milk, very low in bacteria, were inoculated with sour milk in such a way that the number of bacteria added would decrease from tube to tube in any one series. The milk was stored at approximately 8°C. and after ninety-six hours the acidity of the milks was determined. The results of two such trials are presented in table 6.

It is to be noted from table 6 that the correlation between the reduction time and the development of acidity was perfect. It is to be remembered that lactic bacteria predominated in the milk used in these trials. In other words, the bacterial flora was much less complex than that of ordinary market milk.

In samples of ordinary milk not only acid-forming, but also non-acid-forming and alkali-forming bacteria will be present. There would be wide variations in rapidity of growth of the different kinds at varying temperatures. Such complex relations would probably destroy to a considerable extent the correlation between acid development and reduction time. In order to collect some information concerning this point, acid and reduction tests were run on samples of market milk when fresh, and also after storage for twenty-four and forty-eight hours, at approximately 10°C. In table 7 the samples were ranked according to their reduction time when fresh, the sample reducing most rapidly being placed first.

It is to be noted that the acidity has developed more rapidly in the samples of milk which showed a short reduction time when fresh, and in a general way there is a correlation between reduc-

TABLE 6

The relation of the keeping properties of milk to reduction time

SAMPLE	TRIAL I	
	Reduction time	Acidity after ninety-six hours at 8°C.
	minutes	
1	150	0.57
2	186	0.56
3	217	0.51
4	258	0.44
5	303	0.33
6	340	0.28
7	355	0.24
8	371	0.22
9	403	0.20
10	411	0.19

TABLE 7

The relation of acidity and reduction time in milks after varying periods of storage

SAMPLE	ACIDITY AND REDUCTION TIME AFTER 0 HOURS AT 10°C.		ACIDITY AND REDUCTION TIME AFTER 24 HOURS AT 10°C.		ACIDITY AND REDUCTION TIME AFTER 48 HOURS AT 10°C.	
	per cent	*minutes*	*per cent*	*minutes*	*per cent*	*minutes*
56	0.222	125	0.261	10	0.344	1½
30	0.166	205	0.205	11	0.355	2
369	0.188	305	0.194	55	0.210	7
59	0.188	340	0.194	265	0.205	11
86	0.188	360	0.200	260	0.200	22
130	0.210	360	0.210	295	0.216	22
354	0.216	400	0.228	49	0.235	5
135	0.166	400	0.183	335	0.177	160
356	0.188	475	0.188	335	0.177	75
359	0.194	503	0.188	355	0.177	90
357	0.194	510	0.200	385	0.222	15

tion time and keeping quality if the latter is to be measured by acid production. There are, however, certain deviations from this correlation; for example, sample number 135 has shown practically no increase in acid during the storage period, while

the reduction time has decreased. The same is true of samples 356 and 359. In other words, in these samples the effect of acid-forming bacteria if they were present, has been overbalanced by alkali-forming organisms. The latter would, of course, exert an effect on the reduction time. They would not, however, influence the keeping quality as measured by acid development.

It seems from the results obtained that the reduction test will reflect only in a very general way the keeping quality of a sample of milk. It is undoubtedly true that the other methods for the examination of milk, such as the plate culture method and the direct microscopic examination indicate quite as well as the reduction test, the keeping quality. On account of the great complexity of bacterial flora in milk, it seems impossible for the keeping quality to be determined by any one test, or probably by any group of tests.

Directions for making the reduction test

A stock solution of medicinal methylene blue or that recommended for use in staining bacteria is prepared by dissolving one part of the crystalline dye in 2000 parts of water. A portion of this solution is further diluted shortly before use until the concentration is one of the dye to 20,000 of water. One cubic centimeter of this solution in 10 cc. of milk gives a dilution of slightly over 200,000. The solution should not be filtered since this will remove a considerable portion of the dye, especially is this true with dilute solutions.

Tubes of approximately the same diameter and of such a size that 11 cc. will not fill them over one-half full are used. The samples should be collected with the same care as for any bacteriological examination. The tubes should be clean and steamed or boiled shortly before using. It is unnecessary to sterilize them. The 10-cc. sampling pipette should be clean and should be rinsed at least three times with boiled water between samples. After the addition of the dye the contents of the tube should be mixed by closing the tube with the thumb or palm and inverting once or twice. Wiping the moistened area of the hand with a clean

towel will be sufficient prevention of contamination from sample to sample.

A water bath which can be kept at 38°C. in which the tubes can be placed is the only apparatus needed, other than the tubes and pipettes.

The frequency of observation will be determined by the number of grades into which one wishes to divide the milks. There will be little use under ordinary conditions of extending the period of observation over six hours.

Reading the test

It is usually thought that the distribution of bacteria in a recently mixed sample of milk is uniform, in other words, that any small quantity will be an exact duplicate of another sample. There are a number of reasons for believing that this is not true: especially in milk of low bacterial content, among them is the appearance of some of the milk in the reduction test. In the great majority of cases, the color will disappear uniformly from all parts of the milk. In other cases the color may disappear in an uneven way. A small zone or area may lose the color before there is any apparent reduction in other zones. In still other cases the color may persist in the lower layers of the tube after the upper part is completely decolorized. The more common occurrence is the persistence of the color at the top. Vitoux (1920) in his description of the reduction method as used in Holland states that the upper one-fourth of the tube should be disregarded in reading the test. Another method, less convenient in practice, is to take as the end point, the time when no blue color is to be noted after the milk has been mixed. The slight amount of unreduced dye in the surface layer will not be noticeable when mixed with the entire quantity of milk. Again, the color may be reduced to such a point that the milk appears white except when compared with a control tube of milk when a distinct bluish tinge will be noted. This residue of color may persist unchanged for a considerable period. We have not been able to relate this to any factor. It is probable that it is of small importance.

SUMMARY

The importance of the bacterial content of milk in determining its quality is discussed and the need of some simple and inexpensive method for the bacteriological analysis of milk is presented. The methylene blue reduction test seems to meet the need.

The literature treating of the reduction test has been reviewed and a study has been made of a number of factors which influence the results obtained with the test. In the work herein reported attention has been directed to the influence of the concentration of the dye, to the age of the solution of the dye, and to the source or brand of the methylene blue.

The influence of temperature on the reduction time has been investigated.

The direct dependence of the test on the vital activities of the cells is shown by the effect of heating the milk to relatively low temperatures and by the effect of antiseptics.

The relationships which may exist between different groups of bacteria are discussed and data are presented to illustrate the effect of these relationships in influencing the reduction time.

The reducing action of different groups of milk bacteria has been studied as well as the relation of reduction time to keeping quality.

Directions for making the test are given.

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THE COPPER CONTENT OF COWS' MILK

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A review of the literature will reveal many records of the presence of traces of common metals in animal and vegetable substances, although little or no attention is given to these elements in the majority of the published analyses. Obviously, the circumstances governing the analysis of such substances rarely warrants qualitative or quantitative determinations for these minute amounts of metal. This is especially true because, with the exception of iron, it is not known whether their presence is merely accidental or whether they are necessary prerequisites for certain functional activities.

Zinc and copper have been reported in measurable amounts in numerous food substances, and by correlating the results obtained by different investigators it is not difficult to account for their presence in the animal organism. There appears to be authentic data from which it is possible to trace these elements—particularly copper—back to foods of vegetable origin and even to the soil upon which the plants were grown. From the examination of 140 samples of soil Maquenne and DeMoussy (1) found amounts of copper varying from 1 to 250 mgm. per kilogram of earth. They also report copper as a normal constituent of many edible plants, and furthermore, it is stated that this metal is present in all parts of the plant. In many instances the amount of copper was found to greatly exceed that of the soil in which the plant was grown. Guerithault and Maquenne (2), using a somewhat different method of analysis but one for which a high degree of accuracy is claimed, state that copper is found regularly in many digestible substances of vegetable origin. The following table compiled from their data shows a wide range of vegetable products in which copper was found in significant amounts.

Fleurent and Levi (3) have extended these investigations to other vegetable and animal substances. For milk they report 1.4 mgm. of copper per liter; for egg yolk 20 mgm. per kilogram of dry substance; and for horse flesh 22 mgm. per kilogram of dry substance. Rost (4) reports the presence of zinc and copper in measureable amounts in the flesh and organs of various animals, in milk and blood, and in human excreta. Zinc and copper are

TABLE 1

Copper content of digestible substances of vegetable origin

(Guerithault and Maquenne)

MATERIAL	Cu PER 100 GRAMS ASH	Cu PER KILOGRAM OF DRY MATERIAL	Cu PER KILOGRAM OF FRESH MATERIAL
	mgm.	mgm.	mgm.
Sorrel (leaves)	20.7	27.5	2.4
Spinach (leaves)	9.0	18.3	1.8
Lettuce (leaves)	18.0	36.3	2.0
Carrots (roots)	18.0	17.6	2.2
Turnips (roots)	36.0	29.7	3.0
Radishes (roots)	33.0	52.7	3.8
Mangels (roots)	26.6	24.5	3.2
Potatoes (tubers)	15.0	7.7	1.9
Pumpkins	12.0	10.9	1.1
Apples	11.5	7.4	1.2
Pears	19.6	12.9	2.2
Beans	25.4		10.0
Peas	16.9		7.2
Soy Beans	17.3		9.0
Wheat	18.0		7.2
Rye	19.3		7.5
Barley	14.6		6.5
Oats	22.2		17.1
Maize	12.3		6.8

commonly associated together in these substances, and from the data presented zinc was usually found in greater amounts than the copper. It is concluded that these elements are ingested with the food, absorbed from the gastro-intestinal tract, stored in the muscles and in the liver and finally pass into the body excretions and secretions. Thudichum (5) was the first to report the presence of copper in the human brain. Recently Bodansky (6)

has substantiated his observations by quantitative determinations of the copper in the brains of four adults and one foetus five months old. The amount of copper per kilogram of adult brain was found to vary from 3.6 to 6 mgm., whereas the amount found in the fetal brain was 6.8 mgm. The presence of copper and zinc in the tissues of certain marine organisms has been known for a long time and the recent works of Rose and Bodansky (7), and Hiltner and Wichman (8) have furnished further data relative to the copper content of these organisms. Data recently published by Birckner (9) showing the zinc content of many plant and animal substances, but particularly milk, seems to leave no doubt but what this metal is present in measurable quantities in many common foods; determinations for copper were not included in this investigation. Bertrand (10), quoting the work of several investigators states that meat and milk contain 0.50 mgm. of copper per kilogram. (The original article to which reference is made has not been found by us.)

EXPERIMENTAL

Although references to the presence of copper in cows' milk have been found in the literature, the data are meager. The purpose of this paper therefore, is to furnish data showing the variations in copper content of cows' milk, freshly drawn, and after exposure to metallic copper as might occur in milk-handling establishments. The data have been collected over a period of two years in connection with researches on desiccated milk products.

The estimation of copper in milk

The principle difficulty encountered in determining the amount or even the presence of minute traces of copper in milk lies in the selection of method which is sufficiently delicate to detect slight variations without resorting to the use of large quantities of material. Many of the methods commonly used for the determination of copper in foods, while reliable in their specificity for copper and in their quantitative measurement of as low as 1 to 5 mgm., are of no value for indicating amounts as low as 0.1 or

0.01 mgm. Some of these methods however, were used as preliminary tests upon large quantities of freshly drawn milk or milk powder known to be uncontaminated by copper.

The method described in detail by Rose and Bodansky (7) with suitable modifications for liquid milk, yielded sufficient copper sulphide for the positive identification of copper in freshly drawn milk. Qualitative tests for copper on the concentrated liquid from which no more sulphide could be precipitated showed that slight traces of copper were still present. Therefore while the method served to indicate the presence of copper in natural milk, it was not considered sufficiently delicate for the correct measurement of the small amount present.

The potassium ferro-cyanide method was likewise used as a preliminary test for the positive identification of copper in freshly drawn milk. The method was applied to milk ash from which the iron had been removed, and in the presence of traces of zinc salts. A distinct pink coloration was formed indicating the formation of cupric ferro-cyanide. After a period of time the pink color disappeared with the formation of a small amount of dark brown precipitate. In view of the observations of Maquenne and Demoussy (11) who claimed that this reaction is sufficiently sensitive to detect from 1 to 1.5 mgm. of copper per liter, it was considered that these results furnished further confirmatory evidence of the presence of traces of copper in uncontaminated cows' milk.

As further evidence of the presence of copper in milk, 200 grams of milk powder (equivalent to approximately two liters of liquid milk) known to be uncontaminated by copper were examined by the tentative method for determining copper in foods as described in "Methods of analysis of the Association of Official Agricultural Chemists" (12). Since this method is dependent upon the precipitation of sulphide, preliminary tests were made to determine its effectiveness in measuring small amounts of copper in solutions of known copper content. The results of these tests show that the method was unsatisfactory for the correct measurement of copper in quantities less than 1 mgm. From the 200-gram sample of milk powder, sufficient copper sulphide was precipitated however, to permit the identification of the metal.

The method which was found to be most satisfactory for such small amounts of copper as are found in milk, and one which at least gives comparative results without excessively tedious manipulations, is the potassium ethyl xanthate method described by Scott and Derby (13). The method is especially recommended for detecting traces of copper in salts crystalized in copper containers. In addition to its high degree of sensitiveness, it has the advantage that small quantities of iron, lead, nickel, cobalt, zinc and manganese do not interfere. The details of the method as applied to milk follow:

The method is based upon the fact that small amounts of copper react with potassium ethyl xanthate to produce a yellow color. The intensity of the yellow color is in direct proportion of the amount of copper present. When large amounts of copper are added to the xanthate reagent a yellow precipitate of copper xanthate is produced.

Stock solution of copper sulphate: 3.9283 grams of $CuSo_4 \cdot 5H_2O$ are dissolved in water and made up to 1000 cc. One cubic centimeter is equivalent to 0.001 gram of copper.

Standard copper sulphate solution: 10 cc. of the stock solution are diluted to 1000 cc. with distilled water. One cubic centimeter is equivalent to 0.00001 gram of copper.

Potassium ethyl xanthate solution: One gram of potassium ethyl xanthate is dissolved in 1000 cc. of water. This solution should be stored in an amber colored glass stoppered bottle.

Method: 50 to 100 cc. of milk are evaporated to dryness in a silica or porcelain evaporating dish and ignited with a low red heat until a white ash is obtained. Dissolve the ash in the least possible amount of concentrated hydrochloric acid, add about 10 cc. of copper-free distilled water, heat to boiling and filter. If a residue remains on the filter it should be ashed, dissolved in concentrated hydrochloric acid and added to the first filtrate. To the dissolved ash add an excess of concentrated ammonium hydroxide and filter. Wash the precipitate with distilled water. The precipitate is then dissolved in warm dilute hydrochloric acid and re-precipitated with ammonium hydroxide, again filter and wash. The combined filtrates are boiled to drive off excess ammonia. When no more ammonia can be detected, cool, add one drop of concentrated hydrochloric acid followed by 2.5 cc. of 0.5 N sodium hydroxide, and 8 cc. of 0.1 N hydrochloric acid. The solution should be

of neutral reaction at this point, but under no circumstances must it be alkaline. Merely driving off the excess ammonia leaves a slightly alkaline solution which if added to the xanthate reagent causes low results due to the gradual destruction of the color formed by the copper.

The solution containing copper, treated as described above is poured into a 100-cc. Nessler tube, 10 cc. of the potassium ethyl xanthate reagent added, and water added to bring the volume up to the mark. To a series of similar tubes containing 50 to 60 cc. of water add 10 cc. of the xanthate reagent. Then from a burette add to the first tube 0.5 cc. of the standard copper sulphate solution; to the second tube 1 cc. to the third tube 1.5 cc., etc. Add sufficient copper-free water to each tube to bring up to the mark. Compare the tube containing the unknown with the tubes containing the known amounts of copper. If the color of the unknown solution is between two of the standards, gradually add more of the standard copper sulphate solution to the tube containing the lower amount of this standard solution. For example, if the color of the unknown sample falls between the 1-cc. and 1.5-cc. standards add the standard copper solution a drop at a time to the 1-cc. tube until the color in the tube containing the unknown and in the tube to which more of the standard has been added match in intensity. Record the total amount of standard copper sulphate solution used. From this figure the percentage, milligrams per liter, or parts per million of copper in the original sample can easily be determined.

It is necessary to test all reagents for copper and if detectable amounts are found blanks should be run and proper corrections made.

The reliability of the potassium ethyl xanthate method can be shown best by the figures in the following tables. In table 2 is shown the copper found in 10 cc. volumes of water to which known amounts of copper had been added. Samples containing the different amounts of the metal were analyzed by two different analysts who had no knowledge whatsoever of the amounts of copper present. In table 3 is shown the amount of copper recovered from a sample of milk to which definite amounts of the metal in the form of a common copper salt had been added. The amount of copper present in the original sample was known to the analyst but he did not know the amount of metal which had been added. From the results of these determinations it appears

that the method can be considered fairly reliable for copper in milk to as low as 0.005 mgm. Since this amount is considerably less than will be shown to have been present in the samples examined, it is reasonable to assume that the results hereinafter recorded have not been appreciably affected by inadequacies of the method used.

Amount of copper in freshly drawn cows' milk

In order to determine the copper content of milk drawn directly from the cow, several samples from different animals in different

TABLE 2*

Copper in aqueous solution as determined by the potassium ethyl xanthate method

SAMPLE	AMOUNT OF COPPER PRESENT	AMOUNT OF COPPER FOUND (NO. 1)	AMOUNT OF COPPER FOUND (NO. 2)
	mgm.	mgm.	mgm.
1	10	10	Not determined
2	5	5	5
3	1	1	1
4	0.5	0.5	Not determined
5	0.1	0.1	0.09
6	0.02	0.02	0.015
7	0.002	0.002	Not determined
8	0.001	0.001	0.001
9	0.0005	Trace or none	None

TABLE 3*

Copper in milk as determined by the potassium ethyl xanthate method

SAMPLE	AMOUNT OF COPPER IN 100 GRAMS MILK	AMOUNT OF COPPER ADDED	TOTAL AMOUNT OF COPPER PRESENT	AMOUNT OF COPPER FOUND
	mgm.	mgm.	mgm.	mgm.
1	0.04	0.02	0.06	0.065
2	0.04	0.005	0.045	0.050
3	0.04	0.005	0.045	0.045
4	0.04	0.004	0.044	0.044
5	0.04	0.003	0.043	0.040
6	0.04	0.002	0.042	0.045
7	0.04	0.002	0.042	0.040
8	0.04	0.001	0.041	0.040
9	0.04	0.001	0.041	0.045
10	0.04	0.0005	0.0405	0.040

* Indebtedness to Mr. D. I. Stadden of the Laboratory Staff for part of the analyses shown in these tables is hereby acknowledged.

herds were milked directly into the glass containers and at no time were they allowed to come in contact with metal vessels.

TABLE 4

Copper content of freshly drawn milk

SAMPLE	HERD	COW	COPPER PER LITER OF MILK	RATION
			mgm.	
1	I	1	0.75	All cows in herd I received corn, oats, oat straw, and corn stover
2	I	2	0.65	
3	I	3	0.65	
4	I	4	0.45	
5	II	1	0.50	All cows in herd II received corn-meal, wheat bran, oats, ensilage, and hay
6	II	2	0.45	
7	II	3	0.60	
8	II	4	0.55	
9	III	1	0.55	All cows in herd III received oats, gluten, cotton seed meal, and ensilage
10	III	2	0.60	
11	III	3	0.70	
12	III	4	0.80	
13	IV	1	0.30	Ration not recorded
14	IV	2	0.60	
15	IV	3	0.55	
16	V	1	0.60	Ration not recorded
17	V	2	0.20	
18	V	3	0.30	
19	VI	1	0.40	Ration not recorded
20	VI	2	0.50	
21	VI	3	0.30	
22	VII	Mixed sample from 5 cows	0.60	Fresh grass only
23	VII	Mixed sample from same 5 cows	0.40	Highly concentrated with straw as roughage
Average all samples.....			0.521	

The ration being fed at the time the samples were taken was noted whenever it was possible to obtain this informaton. The

results obtained from these determinations are shown in table 4 from which it is evident that a small amount of copper is a regular constituent of uncontaminated cows' milk.

Other analyses of aliquot samples of milk received at milk-receiving plants which buy directly from farmers, and which represented the milk from several hundred cows were made at different times for a period of one year. Of 22 such samples analyzed the maximum copper content was found to be 0.6 mgm. per liter; the minimum 0.4 mgm. per liter; and the average 0.45 mgm. per liter. There was no consistent difference in the copper content of milk produced during the season of pasture feeding as contrasted with the milk produced during the season of stall feeding. It is believed that these results taken in conjunction with those obtained from individual cows represent averages which are applicable to all normal milk. In interpreting these results however, it is to be remembered that larger amounts of copper may be found occasionally in milk which has been exposed to copper utensils during handling and processing before it reaches the consumer.

Copper taken up by milk during handling in milk plants

Several series of analyses have been made in order to determine the amount of copper taken up by milk under conditions to which it might be subjected in passing through various processes commonly employed in milk-handling establishments. Table 5 shows the results obtained in a factory equipped throughout with the common sanitary type milk pipe (tin-lined copper pipe) and with glass enameled tanks for storage purposes or for use in pasteurizing. The entire volume of milk received passed through about 200 feet of sanitary pipe, which upon close inspection was found to have but a very slight amount of exposed copper at a few places where the tin had been worn from the pipe. In its journey through the pipe lines the milk passed several bronze or brass valves, tees and unions very few of which were completely covered with tin. From the time the milk entered the factory until it was ready to leave the only opportunity for taking up

copper was during its passage through the valves, tees, etc., to which reference has just been made. The results given in table 5 show that under the conditions just described little or no copper was taken up by the milk while in the factory. Similar results are to be expected in other plants where equipment is kept in a similar condition.

In contrast with the results obtained where the minimum copper surface is exposed to the milk are those which were found on occasions where the copper content had increased from the average figures of 0.4 to 0.5 mgm. per liter up to as high as 2 mgm. per liter, for no apparent reason other than as a result of an appreciable area of exposed copper in the pipes from which the tin had been worn off. While the maximum figure of 2 mgm.

TABLE 5

Copper content of milk after passage through a well equipped factory

(Results expressed as milligrams per liter)

SERIES	COPPER IN MILK UPON ARRIVAL AT FACTORY	COPPER IN MILK AFTER PASSING THROUGH FACTORY	AMOUNT OF COPPER TAKEN UP IN FACTORY
	mgm.	*mgm.*	*mgm.*
I	0.60	0.75	0.15
II	0.45	0.45	None
III	0.60	0.60	None
IV	0.45	0.45	None

per liter was found in milk after exposure to the conditions just referred to, there were numerous samples which showed a much smaller amount of copper taken up after passage through pipes which could not be considered in a perfect condition, but which were worn to a greater extent than those from which the results of table 5 were obtained. Even though it is apparent that sanitary pipes from which tin has been worn off are a source of added copper in milk, the amount taken up in this manner is believed to be slight as compared with that which may be taken up under other conditions. Even with sanitary pipes which appear to be adequately covered with tin, a very important source of added copper has been found to be the accumulation of soluble copper salts in crevices and on rough surfaces where the bronze

or brass unions, tees, etc., are joined to the tin-covered pipes; the ground surfaces of the valves may under certain conditions become an important source of such material. Obviously, milk passing over these accumulations of soluble copper compounds will take up more copper than by passing over the clean metal of an unprotected copper pipe. Strict sanitary measures must be

TABLE 6

Copper taken up by milk by storing or heating in contact with the metal

SAMPLE	TREATMENT	AMOUNT OF COPPER ORIGINALLY IN MILK	AMOUNT OF COPPER IN MILK AFTER TREATMENT	AMOUNT OF COPPER TAKEN UP BY MILK
		mgm. per liter	*mgm. per liter*	*mgm. per liter*
1	Held in contact with copper pipe twelve hours at 45°F.	0.60	0.96	0.36
2	Heated to 150°F. for two hours in contact with copper pipe.	0.60	5.00	4.40
3	Held in contact with copper pipe twelve hours at 45°F.	0.70	1.00	0.30
4	Heated to 150°F. for two hours in contact with copper pipe.	0.70	2.50	1.80
5	Held in contact with copper pipe twelve hours at 45°F.	2.00	3.00	1.00
6	Heated to 150°F. for two hours in contact with copper pipe.	2.00	4.40	2.40
7	Held in contact with copper pipe twelve hours at 45°F.	1.00	3.20	2.20
8	Heated to 150°F. for two hours in contact with copper pipe.	1.00	5.00	4.00
9	Held in contact with copper pipe twelve hours at 45°F.	0.60	2.80	2.20
10	Heated to 150°F. for two hours in contact with copper pipe.	0.60	8.00	7.40

used regularly to prevent the accumulation of copper compounds in such places.

The copper content of milk as affected by condensing in copper vacuum pans has been shown to be measurably increased, but not to the extent which might be expected in view of the intimate contact between the milk and the copper. Samples of milk which had been condensed at a temperature of 110° to 120°F.,

and which were in contact with the copper condensing pan for a period of one and one-half to two hours were diluted back to the same concentration as the original milk and the copper content determined. The milligrams of copper per liter found in each of six samples follows: No. 1, 3.5 mgm.; no. 2, 1.0 mgm.; no. 3, 0.9 mgm.; no. 4, 1.3 mgm.; no. 5, 1.1 mgm.; no. 6, 3.5 mgm. Considering 0.52 mgm. of copper per liter as the average amount found in normal milk, the increase in the condensed milk samples has varied from 0.38 mgm. to 2.98 mgm. per liter.

The heating or storing of milk in copper containers or in contact with metallic copper materially increases its copper content. The results shown in table 6 illustrate the extent to which this metal may be taken up by milk under the conditions indicated. In each instance the area of copper and the volume of the milk were the same. The average amount of copper taken up by milk after holding for twelve hours at a low temperature was 1.21 mgm. per liter; the average amount taken up after heating for two hours at 150°F. was 4 mgm. per liter.

SUMMARY

Copper was found to be a normal constituent of freshly drawn cows' milk. The amounts found in the milk of individual animals varied from 0.2 to 0.8 mgm. per liter. The average amount found in 23 such samples was 0.52 mgm. per liter. On the basis of results from several samples representing mixed milk taken during periods of pasture feeding and stall feeding, there appears to be no difference in the copper content of milk as affected by the two different types of ration. The maximum amount found in these samples was 0.6 mgm. per liter and the minimum was 0.4 mgm. per liter.

The potassium ethyl xanthate method proved to be the most satisfactory method for the quantitative measurement of the small amounts of copper found in normal milk. Tests to determine the reliability and delicacy of this method show that as low as 0.005 mgm. could be measured with a fair degree of accuracy. Other methods however, were used to advantage for the qualitative detection of copper in large quantities of milk.

The amount of copper in milk may be measureably increased by storing or heating in the presence of metallic copper. Slight increases in the copper content of milk may also result from the passage of milk through the sanitary type copper pipes from which the tin has been worn off. Copper taken up from this source however, is not as great as would result from the formation of copper compounds around brass or bronze fittings as a consequence of poor sanitary practices.

The significance of minute amounts of copper in plant and animal tissues is as yet unknown. Its presence in milk, particularly when taken up as extraneous contamination may prove to be significant in connection with the high susceptibility of the antiscorbutic vitamine to oxidation. In fact, Hess (14) has already reported the destruction of this vitamine by accelerated oxidation as a result of pasteurizing milk in a copper vessel.

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METHODS OF MEASURING THE VOLUME OF CREAM ON MILK

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In the city trade the appearance and volume of the cream on the milk as delivered to the consumer is a matter of considerable commercial importance. This has long been recognized by the milk industry and different methods of measuring the cream have gradually grown up in the different plants. There is, however, an almost complete lack of literature on this subject.[1] The present publication records an attempt to bring together and study these various methods. Finding that they were not satisfactory for the study of the influence of plant operations on the volume of cream, there has been developed a simple and accurate method of measurement which has been found readily applicable under working conditions in commercial milk plants.

METHODS OF MEASURING THE VOLUME OF CREAM

The simplest method of estimating the volume of cream and probably the one earliest used consists in standing bottles beside each other and comparing the depth of the cream layers. Variations are discernible in this way provided the two bottles are of the same size and shape. While there is much to be said in favor of this method it is too crude to be of much service in determining the exact volume of the cream or in tracing the factors which combine in producing the final cream layer which attracts or disappoints the prospective customer.

[1] A more extended discussion of this matter may be found in Ill. Agr. Exp. Sta. Circular 249, 1921.

Measuring from the top of the bottle

A simple test and one much used by dealers is to measure the distance from the top of the bottle to the line dividing the cream from the milk. This was the test applied by Farrington and Russell (1) in their original study of the applicability of 140°F. to the pasteurization of milk. Such measurements do not give the true depth of the cream layer since the bottom of the bottle cap is approximately $\frac{1}{4}$ inch below the top of the bottle.

Measurements of the depth of the cream layer in the neck of bottles are comparable when made in bottles of the same size and shape and with equal depressions for the bottle cap. While it would seem a simple matter for any milk company to keep a supply of uniform bottles, such is not the case. Comparison between measurements in the bottles of different companies is further complicated by the fact that some companies are using narrower and taller bottles which increase the depth of the cream layer.

Marking the cream line and determining the volume

Where measurements must be made of the cream which has already risen in the bottle probably the most accurate available method is to mark the line between the cream and the milk and determine the volume of each. If the bottle is dry on the outside the cream line may be easily marked with a colored wax pencil. A finer line may be made with a brush and ink. Where a brush is not at hand or where the bottle is moist a file may be used.

The magnitude of the error in thus determining the amount of cream present may be tested by repeatedly measuring the volume above the mark in the same bottle. The results of ten such successive measurements in each of a dozen milk bottles where the cream line was marked by a fine ink line showed variations in the determinations of each bottle ranging from 4 to 12 cc. These same variations, expressed in terms of cream percentage, range from 0.4 to 1 per cent, with an average variation of 0.7 per cent.

Measuring the depth of the cream layer in glass tubes

It has long been the custom in making laboratory tests of the creaming ability of milk to put the milk to be tested into graduated cylinders. Cylinders with a capacity of 100 cc. have been most frequently used. While this method is well suited to the laboratory study of a few samples it is not well adapted to handling a large number of samples in a milk plant. Not only are such glass cylinders awkward to transport and store during the period in which the cream is rising but the first cost is heavy and the breakage considerable.

Hammer and Hauser (2) modified this plan in their studies by using large test tubes (size not stated) which they filled with milk to a depth of 6 inches. They measured the depth of the resulting cream layer in sixteenths of an inch and recorded it on a percentage basis, the depth of the cream layer being compared with the depth of the cream layer on a control sample of raw milk. The use of percentages in expressing the results of these studies was unsatisfactory largely because these percentages were based upon the creaming ability of raw milk.

In his later studies of the creaming ability of milk, Hammer (3) used Nessler tubes filled to a line 9 inches from the bottom. The unit used in recording the depth of the cream layer was the sixteenth of an inch and the readings were recorded to the nearest half unit. The Nessler tubes were accurately made and in many ways well adapted to these tests. While more easily stored and handled than graduated cylinders, like them, they are quite expensive and under the conditions which must almost necessarily accompany the taking of large numbers of samples are easily broken.

THE DEVELOPMENT OF A METHOD OF MEASUREMENT SUITED TO MILK PLANT CONDITIONS

A successful study of the factors affecting the cream layer on a bottle of milk must be conducted largely at the milk plant. A workable method for studying these factors should combine simplicity (so as to permit the taking of a large number of samples) with a high degree of accuracy.

The first step in the present study was the selection of the sample tube. Hammer and Hauser used a large test tube, filled to a depth of 6 inches. This was abandoned by Hammer because of the shortness of the milk column and the consequent lack of sensitiveness of the measurements. There was selected for the present purpose a thick walled, glass, test tube, without lip, 1 inch in diameter and 10 inches long. This is a stock size and therefore not unduly expensive.

The next step was to decide upon the depth of the milk sample. Through a desire to have this comparable with the depth of the milk in the quart bottle, it was arbitrarily placed at 216 mm., approximately $8\frac{1}{2}$ inches. However, as pointed out later, there are decided advantages in choosing a slightly different length.

The desired length is marked on each tube by the use of a suitable gage and a sharp file. A tin tube, slightly larger than the glass tube and of the desired length makes a good gage. The glass tube to be marked is inserted in the tin tube, the file is held against it at the edge of the tin tube, and the glass tube is rotated slightly. In this way the glass tube can be quickly and accurately marked. The danger of the glass tube breaking at the file mark can be practically obviated by keeping the file moistened with turpentine during the marking process. In using these tubes to measure the creaming power of the milk before and after any treatment, it is well to fill three or more tubes from the same sample. Using the average of these readings increases the accuracy of the determination. Where the milk to be tested is well mixed and the tubes are filled quickly from a common sample, the resulting cream layer rarely varies more than 1 mm. in depth.

The temperature at which the samples are held is important. The studies of Hammer agree with commercial experience in suggesting that the temperature at which milk is held is a factor affecting the depth of the cream layer. Accordingly, samples to be compared should be kept at the same temperature.

In cold weather the milk at the receiving vat may be at 34°F., while that in the pasteurizer may be at 147°F. To bring samples to the same temperature, it is well to put the tubes at once into

ice water. The samples quickly take this temperature, and they may then be removed to the bottle storage room, which has a temperature of about 40°F. It would undoubtedly result in a deeper layer of cream in the tubes and more uniform results if the samples could be kept at lower and more constant temperatures. However, where the results from the tubes are to be compared with those from the bottles, there are advantages in holding both at the same temperatures.

CALCULATION OF THE VOLUME OF ROUND BOTTOMED TUBES

One of the advantages of the Nessler tube used by Hammer was the fact that it had a flat bottom. When this flat-bottomed tube was filled with milk and later the cream layer was measured, dividing the depth of the cream layer by the depth of the milk gave the percentage of cream by volume.

With a round-bottomed tube, the percentage of cream cannot be figured in quite the same way because the rounded portion of the tube does not contain as much milk as would a flat-bottomed tube of the same length. A necessary step is to determine the length of a cylinder having the equivalent volume.

In the case of the tubes graduated at 216 mm. the following method was used. Starting at the 216 mm. mark, a distance of 200 mm. toward the bottom of the tube was indicated by a fine ink line. The tube was successively filled to the two graduations and the volumes noted, standardized burettes being used.

The computation of the length of the cylinder having the same volume as the lower 16 mm. of the tube was made according to the following simple proportion:

Volume of 200-mm. portion: 200:: volume of 16-mm. portion : x, where x is the length of the equivalent cylinder.

In connection with studies of the creaming power of milk at various milk plants, separate consignments of tubes of these dimensions were purchased through the regular commercial channels. Sixty-two tubes were selected as representative from four such shipments, and their volumes were determined according to the above method. These measurements showed that these 62 round-bottomed tubes measuring 216 mm. in length

had, on the average, the same capacity as flat-bottomed tubes measuring 212 mm. (The extremes of variation were equal to tubes 1.3 mm. longer and 1.1 mm. shorter than the average.)

When the tubes are filled with milk, there ordinarily develops a cream layer of not more than 30 mm.; this gives a ratio of 1 mm. of cream to about 7 mm. of total length. Under such circumstances the above slight variation in the length of the different tubes is not sufficient to measurably affect the final reading. Accordingly, the volume of these tubes at a depth of 216 mm. may be taken as equivalent to that of a flat-bottomed tube 212 mm. long.

The object of these measurements of the tubes was to provide a basis for converting the measurement of the cream layer, given in millimeters, into percentage of cream by volume. These readings may be thus transformed by dividing the reading by 212, or by multiplying it by 0.47. The conversion of the measurement of the depth of the cream layer into percentage by volume can be even more easily done if the length of the sample tube is shortened by 12 mm., so that each millimeter in depth of cream is equivalent to 0.50 per cent by volume. In this case the tube should be filled to a depth of 204 mm., or 8 inches.

EFFECT OF DIAMETER OF TUBE ON CREAM LAYER

The Nessler tube suggested by Hammer has a diameter of about $\frac{1}{4}$ inch smaller than the one here proposed. The question whether this variation in diameter would have any measurable effect upon the depth of the resulting cream layer was tested in the following manner.

There were prepared 50 of the 1-inch, round-bottomed tubes calibrated at a depth of 216 mm., and a like number of the flat-bottomed Nessler tubes calibrated at a depth of 212 mm. Twenty-five tubes from each set were filled with raw milk, as delivered from the barn, at a temperature above 90°F.; and the remaining 25 tubes from each set were filled with milk just pasteurized at 142°F. for thirty minutes. These tests were repeated on two successive days. The tubes of milk were immediately placed in ice water, and when cool were transferred

to a cooler kept at about 40°F. The depth of the cream layer was recorded in millimeters at the end of twenty-four hours.

The average depth of the cream layer developing on the raw milk on the first day was 28.90 mm. in the 1-inch tubes and 28.94 mm. in the Nessler tubes. The corresponding depth of cream rising on the pasteurized milk were 31.20 and 31.22 mm. These averages are as close as could be expected from an average of two sets of the same tubes. On the second day the cream layer in the 25 1-inch tubes of raw milk averaged 33.70 mm. and in the Nessler tubes, 33.14 mm. The corresponding measurements with pasteurized milk were 31 mm. and 30.90 mm. If the fifty determinations with each kind of tube were averaged, the result would be 30.05 mm. with the 1-inch tubes and 30.09 with the Nessler tubes. The results make it evident that when cylinders differing in diameter from $\frac{3}{4}$ inch to 1 inch are filled with milk to the same depth and held at the same temperature, they will later develop equal layers of cream.

COMPARISON OF VARIOUS METHODS OF MEASURING THE CREAMING ABILITY OF MILK

Attention has already been called to the various methods now in use in milk plants for measuring the volume of cream. A comparison of the results obtained by these different methods is made possible by applying them simultaneously to the same milk. Accordingly bottles were taken from the bottler and immediately placed in the cooler for about twenty hours. The depths of the cream layers were then measured, the cream line was marked, and the volume of the cream and the per cent of cream in each bottle determined. When these bottles were selected at the bottler additional bottles were taken and from them test tubes were filled, the tubes of milk cooled in ice water, and placed in the cooler with the bottles for about twenty hours. The depth of the cream in these tubes and the per cent of cream which these readings represented was also noted. The results of these comparable observations are given in table 1.

From the data in table 1 it is evident that the measurement from the top of the bottle is an inaccurate method of determining

the amount of cream present. Among the nine bottles measuring three inches of cream the per cent by volume present ranged

TABLE 1

Comparison of various methods of cream measurement

SAMPLE NUMBER	MEASURED IN THE BOTTLE				MEASURED IN TUBE	
	Cream depth	Bottle volume	Cream volume	Cream	Cream depth	Cream
	inches	*cc.*	*cc.*	*per cent*	*mm.*	*per cent*
1	$2\frac{14}{16}$	938	98	10.45	22.0	10.38
2	$2\frac{14}{16}$	940	103	10.96	23.5	11.09
3	$2\frac{14}{16}$	936	108	11.54	24.0	11.32
4	$2\frac{14}{16}$	940	110	11.70	25.0	11.79
5	$2\frac{14}{16}$	944	108	11.14	23.0	10.85
6	$2\frac{14}{16}$	929	105	11.18	25.0	11.79
7	$2\frac{14}{16}$	936	109	11.65	25.0	11.79
8	$2\frac{14}{16}$	933	112	12.00	26.0	12.26
9	$2\frac{14}{16}$	931	116	12.46	26.5	12.50
10	3	940	109	11.60	24.5	11.56
11	3	932	113	12.12	25.0	11.79
12	3	942	121	12.84	25.5	12.02
13	3	941	121	12.85	27.0	12.74
14	3	942	121	12.85	26.0	12.26
15	3	930	120	12.90	26.0	12.26
16	3	937	125	13.34	25.5	12.03
17	3	941	130	13.82	26.0	12.26
18	3	939	132	14.06	27.5	12.98
19	$3\frac{1}{16}$	938	111	11.83	25.5	12.03
20	$3\frac{1}{16}$	946	120	12.68	26.0	12.26
21	$3\frac{1}{16}$	941	120	12.75	25.5	12.03
22	$3\frac{1}{16}$	943	122	12.94	27.0	12.74
23	$3\frac{1}{16}$	933	122	13.08	27.0	12.74
24	$3\frac{2}{16}$	946	121	12.79	27.0	12.74
25	$3\frac{2}{16}$	932	121	12.98	26.0	12.26
26	$3\frac{2}{16}$	937	122	13.02	26.0	12.26
27	$3\frac{2}{16}$	940	135	14.36	28.5	13.45
28	$3\frac{2}{16}$	938	125	13.33	27.0	12.74
29	$3\frac{2}{16}$	939	129	13.74	29.0	13.68
Average				12.51		12.16
			Difference,	0.35 per cent		

from 12.46 to 14.06. Sample 18, which measured only 3 inches of cream, actually contained more cubic centimeters of cream than either of the bottles with cream layers measuring $3\frac{3}{16}$ inches.

On the other hand the cream layer on sample 10, which likewise measured 3 inches, contained only 109 cc. of cream, or less cream than sample 4 with a cream layer measuring only 2 $\frac{14}{16}$ inches.

The data here given is not enough to show the range of variation to be expected in measurements of the cream layer from the top of the bottle but enough is given to make it plain that a variation of at least $\frac{1}{4}$ inch is necessary before one can be fairly sure which bottle contains the more cream. It should also be remembered that these measurements were made of the bottles of a single company which was striving to keep a stock of uniform bottles. Where comparisons are made between bottles of different companies the measurements of the cream layer from the top of the bottle are liable to be even more misleading.

The results obtained by marking the bottles and determining the cubic centimeters of cream present, when reduced to per cent by volume, correspond fairly well with similar figures from the test tube determinations. It will be observed, however, that the results obtained from the bottles range more widely than those from the test tubes. It has already been pointed out that average variation in the measurement of the cubic centimeters of cream in a bottle amounts to 0.7 per cent while the ordinary variation in the test tube determinations is less than 1 mm. which is equivalent to 0.47 per cent.

A comparison of the results as given by the two methods for similar bottles shows that in almost all cases the percentage of cream as determined by the test tubes is less than that determined direct from the bottle. The average of all these determinations in table 1 is 0.35 per cent by volume less for the test tube determinations. This variation while real is not very significant because it is only one-half of the normal variation in determining the volume of cream in milk bottles by direct measurement.

SUMMARY

The volume of cream in the milk bottle is important and there are wide differences of opinion as to the factors influencing this volume. This conflict of opinion is largely unsupported by

direct evidence and is primarily due to the lack of a method of measurement which is both accurate and readily applicable under milk plant conditions.

The improved method here described consists in filling round-bottomed test tubes, 1 inch in diameter, to a depth of 204 mm., 8 inches, with the milk to be tested. These tubes of milk are immediately cooled in ice water and when cool are held at 40°F. for approximately twenty hours. The depth of the resulting cream layer is measured in millimeters and each millimeter of cream represents 0.5 per cent of cream by volume. The volume of cream as determined in this way agrees closely with the volume of cream developed in milk bottles under similar temperature conditions.

This method has been extensively tested in milk plants and its advantage lies in the fact that by its use a large number of samples may be collected during a single day, the samples stored compactly, and measurements of the cream made quickly, accurately, and quantitatively.

ACKNOWLEDGMENT

In developing this method and testing its applicability to milk plant problems extensive observations have been made in the milk plants of the Gridley Dairy Company, of Milwaukee, Wis., the Sheffield Farms Company, Inc., of New York City, the Detroit Creamery Company, of Detroit, Mich., the Pevely Dairy Company, of St. Louis, Mo. and the Bowman Dairy Company, of Chicago, Ill. The opportunity thus afforded of testing the method under commercial conditions has added much to its value and acknowledgment is gladly given of the many courtesies extended. While in connection with each of these milk companies there have been a number who by their criticism and suggestions have aided in this and the accompanying studies mention is especially due Messrs. S. M. Heulings, of the Sheffield Farms Company, Inc., and W. D. Dotterer, of the Bowman Dairy Company.

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A WEIGHT-HEIGHT-AGE CURVE AS A MEASURE OF THE STATE OF NUTRITION AND OF GROWTH OF THE DAIRY COW

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The weight of an individual cow at a given age is, by itself, not an index of her condition or state of nutrition. The cow may naturally, by heredity, have a skeleton somewhat below or above the average in size and, therefore, because of the tendency to keep the symmetry between weight and height constant, also weigh less than the average. It is in fact very rare that an individual cow, selected at random, has the weight of an average cow. A ten-month-old Holstein heifer selected at random from our herd was found to differ by 50 pounds from the average weight of our ten-month-old Holstein heifers; and the difference in weight between the heaviest and lightest heifers of this breed and age in our herd was found to be 270 pounds. In other words comparing an average individual heifer under experimental conditions to the average weight of a heifer of the same age as a standard may introduce an average experimental error of 50 pounds due to the individuality of the animal and this may be as high as $\frac{270}{2}$ or 135 pounds, or even higher.

These illustrations indicate the inadequacy of using the average weight by itself as a standard of comparison for the determination of the condition of a cow under experimental conditions and suggests the necessity of adopting some standard of measure which will take into consideration what might be termed as the "hereditary size" of the animal. The ideal hereditary size of each individual, if known, would be the ideal standard of comparison for that individual under the given experimental conditions, in order to determine what effect such experimental conditions have on the animal.

Investigations at this station by Waters, Mumford, Trowbridge and their associates have shown that experimental conditions exert little or no effect on the growth in height at withers. This is strikingly illustrated by figures 1 and 2[1] showing the relative effect of experimental conditions on height and weight on the same group of animals. The effect on weight is profound; the effect on height at withers is practically negligible.[2]

If height at withers is practically independent of experimental conditions it may be taken as an unvarying measure of the hereditary size of the animal at any age. If the relation between height and weight under "normal" conditions is established, it is easy to determine from this established relation, knowing the height, what should be the corresponding weight. This is easiest accomplished by a weight-height curve as shown in figure 3.[3] For every height there is under a given set of conditions, a corresponding definite weight. Knowing the height of the animal under experimental conditions the weight corresponding to this height is found from this curve for "normal" animals and compared to the actual weight. The difference between the two is a measure of the effect of the experimental conditions on the animal.

That there is a definite weight for a given height under "normal" conditions regardless of the hereditary growth capacity, is shown in figure 3. The height and weight of the relatively small Jersey fall on the same line as the weight and height of the much larger Holstein, and this is probably true for all dairy breeds. The relation of symmetry probably holds for all breeds of the same type. The only difference between the different breeds of different hereditary sizes, consists in the dif-

[1] The writers are indebted to Dr. C. R. Moulton, Chairman of the Department of Agricultural Chemistry in this station for figures 1 and 2. Further details concerning these animals may be found in Research Bulletin 43 of this station.

[2] In this connection it is important to note that investigations at this station by Eckles, reported in Research Bulletins 31 and 36, have shown that from birth to maturity the dairy animal increases only 90 per cent in height at withers while in the same period of time it increases, approximately, 1423 per cent in weight.

[3] Figure 3 is based on data in Research Bulletin 36 of this station by C. H. Eckles.

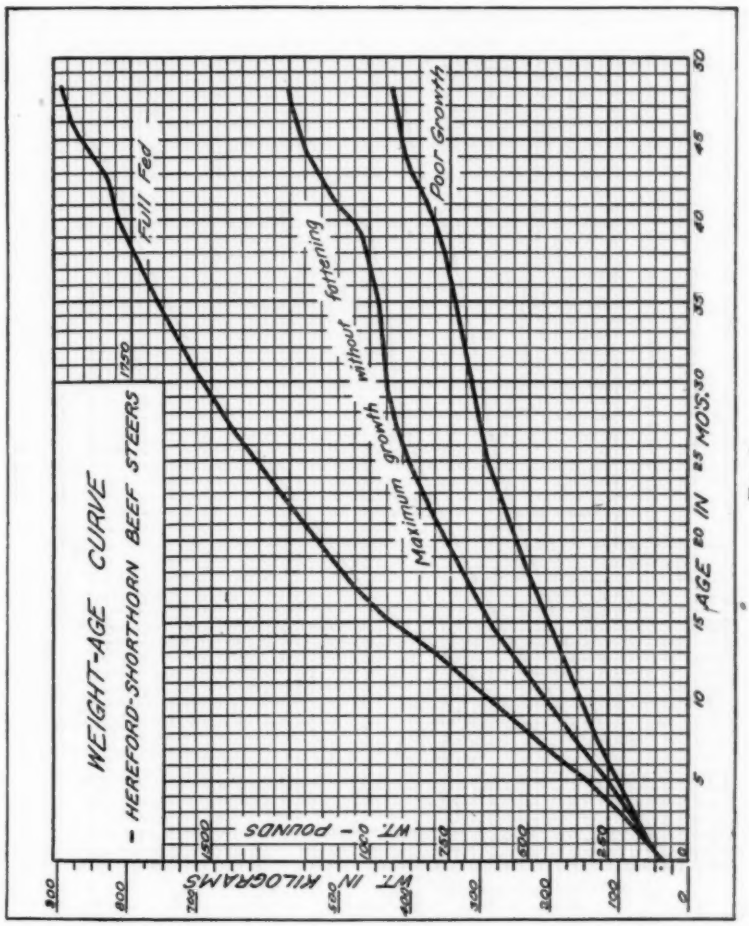
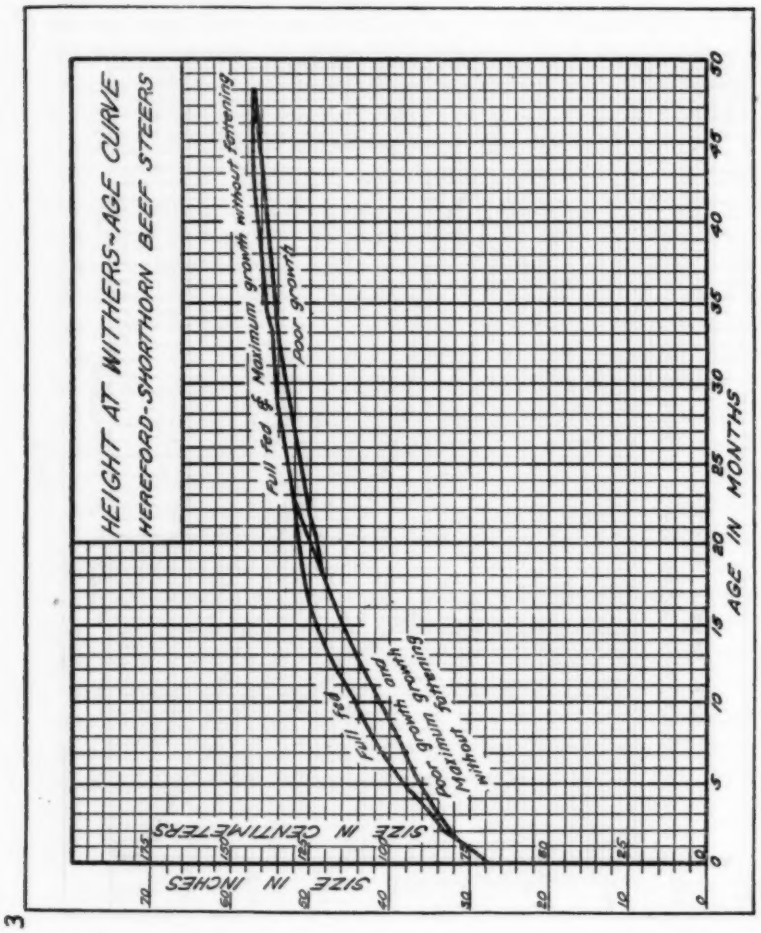

WEIGHT-AGE CURVE
- HEREFORD-SHORTHORN BEEF STEERS
Full Fed
Maximum growth without fattening
Poor Growth
WT. IN KILOGRAMS
WT. - POUNDS
AGE IN MOS.
0
5
10
15
20
25
30
35
40
45
50
1250
1000
750
500
250
100
200
300
400
500
600
700
800
900
1000
1100
1200

FIG. 1


HEIGHT AT WITHERS-AGE CURVE
HEREFORD-SHORTHORN BEEF STEERS
Full fed & Maximum growth without fattening
Poor growth
Full fed
Maximum growth and without fattening
Poor growth and without fattening
SIZE IN CENTIMETERS
SIZE IN INCHES
AGE IN MONTHS
0
5
10
15
20
25
30
35
40
45
50
55
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80
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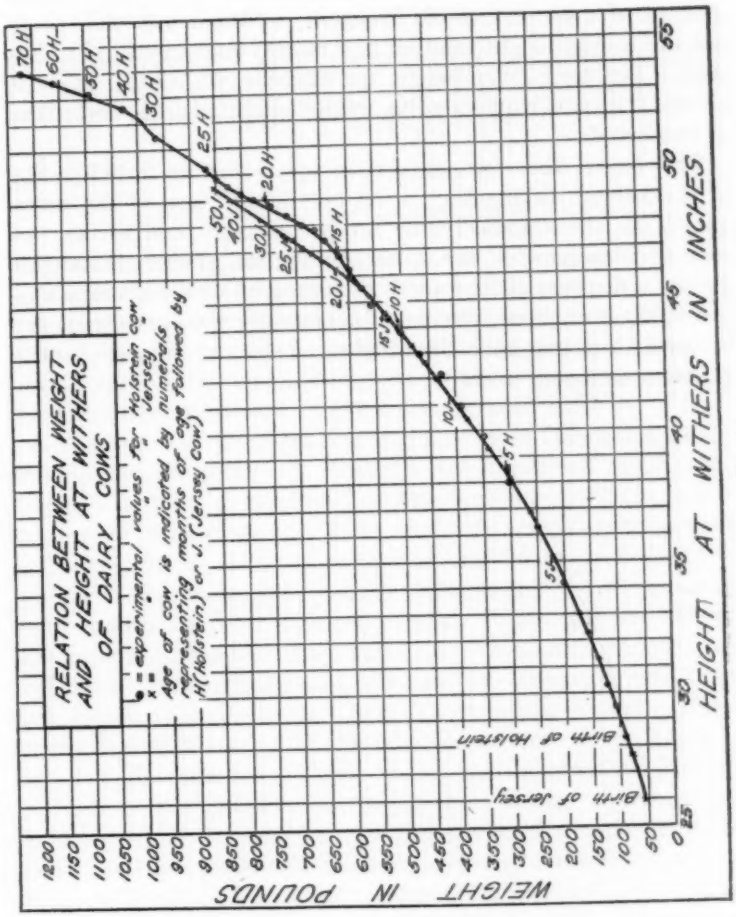
RELATION BETWEEN WEIGHT AND HEIGHT AT WITHERS OF DAIRY COWS
● = experimental values for Holstein cow
× = " " " Jersey
Age of cow is indicated by numerals representing months of age followed by H (Holstein) or J (Jersey cow)
WEIGHT IN POUNDS
HEIGHT AT WITHERS IN INCHES
Birth of Jersey
Birth of Holstein
70H
60H
50H
40H
30H
25H
20H
15H
10H
5H
5J
10J
15J
20J
25J
30J
40J
50J

Fig. 3

ference between the age-height relationship and age. Thus from figure 3, a fifteen-month-old Jersey has the weight-height relation of a ten-month-old Holstein; a fifty-month-old Jersey has the weight-height relation of a twenty-four-month old Holstein. But for a given height, for example, 45 inches, there is always the same corresponding weight of 550 pounds, regardless of the breed.

This curve, therefore, eliminates errors due to individuality of the animal, taking the "ideal hereditary" size of the given animal as the standard unit for its measure and serves not only as a measure of the state of nutrition of the animal but also as a measure of its growth as compared to the average animal of the given breed or race. Incidentally the curve may also be used for determining the weight or height at any age for the Holstein or Jersey cow.

ACTINOMYCES IN MILK WITH SPECIAL REFERENCE TO THE PRODUCTION OF UNDESIRABLE ODORS AND FLAVORS[1]

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To the knowledge of the author it has not been heretofore suggested that actinomyces may cause certain obnoxious tastes and odors in dairy products. The mere presence of these organisms in milk and other foods has scarcely been noted, while their activities are still less known. Only a few references of direct connection to this paper are considered here.

Gratz and Vas (7) described two species of casein-dissolving actinomyces from cheese. Coombs (4) found the number of fungus spores in normal and moldy butter greatly decreased in number during ageing. It is probable actinomyces as well as molds play a part in causing abnormal flavors in butter for the odors and tastes produced by actinomyces are if anything more penetrating than those of molds. Thom (14) has shown that fungus spores find their way into milk mainly from fodder and dust. Some spores pass through the separator and thus gain access to the butter. Conn (3) described several actinomyces-like organisms such as B. arborescens lactis n. sp., Bacillus 138 and Bacillus 184. These organisms imparted to butter made from inoculated milk, undesirable flavors. Moore (10), Dykstra (6), and others have stated the natural habitat of A. bovis, the ray fungus is in the soil, and that the method of entrance to the body is by a cut or abrasion about the head. This organism is not known to be transmitted to the milk. Waksman

[1] Work done at N. J. Agr. Exp. Stas., New Brunswick, N. J. Acknowledgment is due Dr. J. G. Lipman, Director, and Mr. L. S. Riford formerly Assistant Dairy Husbandman for criticism and encouragement during the progress of the investigation.

(16) has established the identity of morphology, cultural characters and biochemical reactions of the so-called saprophytic and parasitic species. Practically the only differences lie in the temperature relations and pathogenicity. He has also shown that while many species grow well in milk as a medium, only some of them cause coagulation and peptonization. Some species which he studied were able to utilize both the carbon and nitrogen of the casein. Conn (2), Krainsky (9), Munter (11), Rossi-Doria (12) and Waksman and Curtis have contributed much of value to the literature regarding this group of organisms.

EXPERIMENTAL

This investigation deals with an outbreak of an offensive taste and aroma developing in milk at a New Jersey dairy. From sixteen to forty-eight hours was necessary for the repulsive taste to develop. At first it resembled somewhat strong turnipy milk later developing the characteristic earthy and bitter-moldy taste and odor. The dairy was well managed and equipped to produce milk and cream of excellent quality. Utensils were sterilized twice daily, the cows were cleaned daily, the bedding was baled straw while the feed consisted of alfalfa, sedge grass and grain. The fodder was grown on low mucky land and had a somewhat earthy odor.

Samples of the milk and cream developing the offensive taste were plated bacteriologically on lactose litmus agar and incubated at 25°C. for several days. The plates were found to be populated largely by actinomyces colonies, the average of the six samples examined being 1,100,000. A sample of cream gave a count of 2,400,000 per cubic centimeter. In these samples no differentiation was made between actinomyces and bacterali colonies. Another sample of cream plated on Czapek's fungi agar (5) and incubated for eight days at 25°C. yielded 1,100,000 actinomyces and 1,000,000 bacteria per cubic centimeter. In still another series of samples the counts were 440,000 actinomyces and 120,000 bacteria. The offensive taste was shown to be due to a biological factor because it required from sixteen to forty-eight hours for the taste to develop after inoculating

sterile or normal fresh milk with very small quantities of the abnormal milk. Such inoculated samples invariably revealed the presence of large numbers of actinomyces after plating on Czapek's agar. This medium gave excellent satisfaction and on it actinomyces develop aereal mycelium and otherwise appear more characteristic than on ordinary peptone-beef with or without sugars. It is believed that the lack of suitable media, the short incubation period usually employed, and the confusion of young actinomyces colonies with bacteria, are probable reasons why this group of organisms in milk and in other food substances, has been largely overlooked.

The trouble gradually subsided during the summer when the cows were receiving fresh feed, but recurred again in the fall. Again the dairy was inspected, samples taken, and the cause of the bitter-moldy taste traced to the large number of actinomyces present. Samples packed in ice and examined bacteriologically after twenty-four hours, showed, 1,000,000 actinomyces and 450,000 bacteria per cubic centieeter for milk, and 1,800,000 actinomyces and 700,000 bacteria per cubic centimeter of cream. Samples of milk from six cows in the dairy were drawn directly into sterile flasks under regular milking conditions, packed in ice and examined after about twenty-four hours. The cows were brushed with a cloth and given clean straw for bedding a short time before milking. Samples of the hay, grain, straw, manure, water and litter, were also taken to the laboratory for bacteriological examination.

Table 1 contains data regarding the milk drawn from three cows of the herd under investigation as well as several other representative herd and market samples for comparison. Czapek's agar with a seven-day incubation at 25°C. was used in this work. The average per cent of actinomyces in the normal milk examined was about 2.5; the minimum, 0, and the maximum 10 per cent. Practically all raw milk contains some of these organisms because of the practical difficulties preventing dust, soil and bedding contamination of the milk. From table 1 it is evident that the milk of three cows in the affected herd contained a sufficient number of actinomyces to give a distinct

TABLE 1

Relative numbers of actinomyces and bacteria in milk

SOURCE OF SAMPLE	TASTE FRESH	TASTE TWENTY-FOUR HOURS	TASTE FORTY-EIGHT HOURS	BACTERIA PER CUBIC CENTIMETER, CONN'S ACTINOMYCES AGAR, FOUR DAYS, 25°C.	ACTINOMYCES PER CUBIC CENTIMETER, CONN'S ACTINOMYCES AGAR, FOUR DAYS, 25°C.	CHARACTER OF SAMPLE IN FOUR DAYS	AMOUNT REQUIRED TO PRODUCE TASTE IN 100 CC. MILK IN TWENTY-FOUR HOURS AT 25°C.
							cc.
Primrose 3rd (affected herd)	Normal	Bitter-earthy	Bitter-moldy, marked	450,000	700,000	Curdling just beginning; intense bitter-pungent taste	1
Florham Fashion (affected herd)	Normal	Oily-earthy	Bitter-oily	50,000	210,000	Curd precipitated; whey clear; slight brown color, very bitter	1
King's Pride (affected herd)	Normal	Normal	Flat-earthy	260,000	650,000	Solid curd, normal souring	10
Mixed milk College Farm herd	Normal	Normal	Slightly sour	500,000	8,000	Normal souring	0
College Farm herd	Normal	Normal	Slightly sour	220,000	3,000	Gassy curd	0
College Farm herd (after sweeping)	Cowy	Normal	Sour with gas	2,200,000	220,000	Gassy curd partly digested	0
Creamery New Brunswick, N. J.	Normal	Normal	Normal souring	390,000	15,000	Normal souring	0

Dairy at New Brunswick, N. J.	Normal	Slightly sour	Sour	400, 000	8, 000	Solid curd, some gas	0
Dairy at Charlotte, N. C.	Normal	Normal	Normal	60, 000	500	Normal curd	0
Dairy at Charlotte, N. C.	Normal	Normal	Normal	120, 000	2, 000	Normal curd	0
Dairy at Columbus, Ga.	Normal	Normal	Sour	280, 000	6, 000	Slightly gassy curd	0
Dairy at Seattle, Wash.	Normal	Normal	Slightly sour	40, 000	3, 000	Firm curd	0

earthy flavor to the milk held at air temperature for twenty-four hours. In thirty-six hours the milk was so offensive to the taste that it would have been impossible to use it. The taste is described as bitter-moldy, but the term "actinomyces taste" is suggested as being more descriptive and correct. In two of the three samples the curd was precipitated in four days with partial digestion; in the third sample no curdling occurred. Of the first two samples above, only 1 cc. was necessary to impart to normal sterile milk an actinomyces taste in twenty-four hours. Counts made by the author on various market milks, show the numbers of actinomyces present is relatively low. The affected herd's water supply contained only 2 actinomyces per cubic centimeter.

Table 2 contains data relative to the numbers of actinomyces in various materials. In order to determine the source of the actinomyces, straw, hay, grain, soil, muck, dust, and manure were examined bacteriologically. This data is found in table 2.

From table 2 it is evident that actinomyces are widely distributed in nature, particularly in dry hay, straw, dust and soil. Muck soils and sod contain more of these organisms than dry fallow or cultivated soils. The spore-like bodies of actinomyces are very light and remain in the air for a long time. This was proven both in the laboratory and in the stable. Petri dishes exposed for thirty minutes after raising dust contained many actinomyces colonies—the proportion of the latter to bacteria being greater after partial subsidence of the dust. Neither ground nor surface waters appear to carry many actinomyces. The hay and straw from the dairy producing the bitter-moldy milk, contained an unusually large number of these organisms and it is extremely likely that dust contamination in the stable before or at the time of milking was responsible for the trouble.

Over 30 types embracing 11 species were isolated and identified from the milk. These same species and many additional ones were identified from soil, hay, straw and dust. Some of the organisms were very slow growing, some produced no aerial mycelium and grew only upon special media and still others produced no characteristic changes in milk. Waksman (15,

TABLE 2

Numbers of actinomyces in various substances

SUBSTANCE	MEDIUM USED	ACTINOMYCES PER GRAM
Ground alfalfa hay	Conn's actinomyces agar incubated seven days, 25°C.	3,900,000
Timothy and clover hay	Conn's actinomyces agar incubated seven days, 25°C.	2,300,000
Redtop and timothy hay	Conn's actinomyces agar incubated seven days, 25°C.	600,000
Chopped wheat straw	Conn's actinomyces agar incubated seven days, 25°C.	390,000
Shredded oat straw	Conn's actinomyces agar incubated seven days, 25°C.	90,000
Dried manure	Conn's actinomyces agar incubated seven days, 25°C.	145,000
Wet manure	Conn's actinomyces agar incubated seven days, 25°C.	1,500
Ground oats	Conn's actinomyces agar incubated seven days, 25°C.	190,000
Ground mixed feed	Conn's actinomyces agar incubated seven days, 25°C.	6,000
Dried soy beans	Conn's actinomyces agar incubated seven days, 25°C.	8,000
Single soy bean seed having musty odor	Ashby's nitrogen-free agar	95,000
Five alfalfa seeds	Ashby's nitrogen-free agar	3,600
Soil sample 1	Conn's actinomyces agar	100,000
Soil sample 2	Conn's actinomyces agar	70,000
Soil sample 3	Conn's actinomyces agar	150,000
Muck soil	Ashby's nitrogen-free agar	350,000
Muck, Farmogerm legume inoculant	Ashby's nitrogen-free agar	1,800,000
Muck soil, Alphano, N. J.	Ashby's nitrogen-free agar	750,000
Grass roots from muck soil	Conn's actinomyces agar	475,000
Average of four water samples	Conn's actinomyces agar	3
Dusty air, five minutes exposure	Conn's actinomyces agar	20,000 (10 per cent of total colonies)
Dusty air, ten minutes exposure in stable	Conn's actinomyces agar	76,000 (16 per cent of total colonies)

16, 17) has published complete descriptions of 41 species. He noted their growth on milk, action on casein and enzyme production. In this particular study the most frequently encountered organisms were closely similar types of the A. griseus group, represented by A. griseus Krainsky. Because of its importance a somewhat complete description of this species is reproduced here. Krainsky's original description is very incomplete.

Actinomyces griseus Krainsky

Czapek's agar. Colonies round, raised, 2 mm. diameter in four days, color water green (G. tint 2).[2] Reverse, sage yellow to lemon yellow (G. Y. tint 2 to O. Y. normal tone) in old cultures. White aerial mycelium, formed early changing to water green color. Powdery colonies. Old cultures become a light fluorescent green color.

Microscopically the colony appears as fine radiating many branched filaments. The latter break up into short chains or rods of conidia each conidial chain being from 1 to 2.2 micra long and from 0.6 to 0.8 micron broad. On staining these show oval spore-like bodies 0.8 to 1.2 micra long by 0.5 to 0.9 micron broad, which are highly refractive and stain with difficulty. They occur in chains, pairs or branching chains preserving the form of the original filaments. Odor of culture, strong actinomyces (musty straw).

Bouillon agar. Abundant but uncharacteristic growth in three days. Colonies round, much raised and white. Aerial appears late, chalky white at first becoming darker with age. Enzymic zone or halo extends around the colony. Medium only very slightly darkened. Reverse color brownish (Y. O. shade 2); odor, moderately strong actinomyces.

Conn's glycerin actinomyces agar (2). Colony diameter in three days about 2.5 mm. and in six days from 3 to 5 mm. Color, white at first, later becoming water green with a gray mycelium in the substratum. Reverse (G. Y. tint 2); old colonies much zonated. Few or no spiral structures in aerial mycelium. Microscopically like Czapek's agar. Hyphae stain well with methylene blue, the conidia appearing in chains within the filament. Growth on this medium is poor and not characteristic when either the glycerin is omitted or the reaction is made neutral.

[2] See color charts accompanying Mulliken's "Identification of pure organic compounds."

Krainsky's calcium malate agar (9). Growth similar though not as vigorous as on Conn's medium, scanty at first then spreading. Aerial mycelium white to gray at first later becoming pale green (Y. tint 2); reverse (O. tint 2).

Starch agar. Growth poor even after ten days; diastatic activity weak.

Gelatin (15 grams in 1000 cc. distilled water). Colony diameter in four days, 1 mm. while in seven days it is 1 to 1.5 mm. with a liquefied pit from 4 to 6 mm. diameter. The growth is grayish and the liquefied portion shows a slight yellow-green fluorescence, becoming darker with age. Aerial mycelium slow to form, scanty, white, with no spiral bodies. Growth more vigorous on acid than on neutral gelatin. Pigment is deeper on neutral media. Color, yellow to golden yellow (Y. to golden Y.).

Potato. Vigorous growth; brownish white, abundant aerial mycelium becoming greenish colored about the seventh day. Odor, moderately strong actinomyces. Potato darkened slightly in three days, the color increasing in intensity with age.

Czapek's solution. In four days the medium assumes a green color and is filled with white flakes. Colonies appear on the surface in from seven to twelve days, they also appear on the bottom and sides of the flask. Odor, moderately strong actinomyces.

Milk. A bitter moldy taste and earthy odor develops in from twenty-four to forty-eight hours. No growth evident macroscopically for about three days, when colonies develop on sides and bottom of flask. Surface colonies appear in from six to ten days, the color is white at first and gray later. In from three to five days the odor becomes very strong and the taste extremely bitter-moldy; the casein is coagulated in from three to seven days, active peptonization then begins and is practically complete in from ten to twelve days. The reaction becomes alkaline.

Nitrate reduction. The reduction of nitrates to nitrites in a peptone-nitrate medium was relatively weak. The transformation of protein into ammonia however was strong.

Indol production. Negative.

Nitrogen transformation. In four days at 25°C., this organism ammonified 3.04 per cent of the total nitrogen contained in whole milk. Under similar conditions, 4.88 per cent of the total nitrogen contained in a 1 per cent casein solution was converted into ammonia. The initial acidity in terms of lactic acid was 0.18 and after four days the reaction was alkaline.

Besides A. griseus, the following species were identified from milk; A. albus Krainsky, A. alboflavus Waksman and Curtis, A. flavus Kriaisky, A. lipmani W. and C., A. albosporeus Krainsky, A. diastaticus Krainsky, A. rutgersensis W. and C., A. bobili W. and C., an organism corresponding in some respects to A. violaceus W. and C. and a slow growing species which formed no aerial mycelium on any media. This organism was very active in milk and was apparently a new species. All of these organisms produce a bitter-moldy taste in milk but the more active species are A. griseus, A. albus, A. rurgersensis and A. lipmani. From twenty-four to forty-eight hours is usually required for the odor to develop, although the taste is evident in a shorter time. It is possible therefore for milk heavily contaminated with actinomyces, to become offensive before it reaches the consumer. Refrigeration materially delays but does not absolutely inhibit the formation of the objectionable taste. The character of the bitter substance is unknown; it is probably due to extracellular enzymes or other soluble bodies elaborated by the organisms during active growth. Usually there is no macroscopic change in milk for several days then more or less complete coagulation of the casein followed by peptonization.

ACTINOMYCES IN OTHER FOODS

To the author's knowledge actinomyces have not been mentioned as a cause of stale, musty or moldy odors and flavors in foods or food materials. It is certain many of the odors and tastes commonly attributed to molds are really due to actinomyces. The author has twice examined musty smelling walnut meats and found the cause to be actinomyces. Both spores (conidia) and mycelium were present and the species resembled closely A. griseus, though not entirely identical with it. In another case, dried fish flakes were found to be stale and musty smelling. Bacteriological examination revealed the presence of both actinomyces and molds. Upon reinoculation however only those flakes inoculated with actinomyces reproduced the

characteristic odor and taste. The mold was therefore deemed of secondary importance, though its penetrating powers were greater than the actinomyces.

Oats, barley, corn and ground cereals often become moldy, particularly when excessive moisture is present. Several species of aspergillus, penicillium and fusarium are often present under such conditions. Thom (13) has reported the presence of many species of molds and actinomyces in normal and spoiled corn meal. The author has examined several samples of oats and barley which contained large numbers of actinomyces, molds, spore-bearing bacteria and B. aerogenes. It is possible actinomyces may play a part in the development of musty odors in grains under certain conditions. Dried eggs often contain many actinomyces and it has been suggested[3] that the moldy odor and taste often encountered in this product may be due to this group of organisms. They thrive well in dry places, are not readily killed by desiccation and may utilize a wide variety of materials for their food. The odors and tastes produced by them are intense and objectionable and easily permeate the substratum in which they are growing, and for these reasons are likely to cause trouble in a variety of products.

SUMMARY

Actinomyces are often present in market milk samples. In normal samples these organisms constituted about 2.5 per cent of the total organisms present. In abnormal samples, particularly those drawn from cows in dusty stables, this figure may reach 50 per cent.

The principal means of entrance to milk are hay, straw, grain, soil and dust raised from these materials.

Actinomyces may under certain conditions cause an obnoxious bitter-moldy taste to develop in milk after some hours storage. The term "actinomyces taste or odor" is suggested to describe the characteristic taste and odor of actinomyces.

[3] Personal communication, Dr. H. W. Redfield, Chief, N. Y. Station, U. S. Bureau of Chemistry.

Two outbreaks of bitter-moldy milk in the same dairy were traced to the presence of large numbers of actinomyces. The source of the contamination was probably hay and straw.

Eleven species were identified from the milk. The two most active species concerned were A. griseus group and A. albus. These organisms grow readily in milk and are able to produce profound changes in the casein and whey. The extremely diffusible and volatile substance which causes the odor and taste so characteristic of actinomyces is not known.

Actinomyces may cause stale, musty and moldy odors in such foods as walnuts, dried fish, cereal grains and possibly dried eggs. They occur together with molds in many foodstuffs, especially those in a dried condition.

In studying actinomyces or in attempting to isolate them from foods or other materials, synthetic media combined with long low temperature incubation should be used. The ordinary methods and media with incubation at 37°C., are unsatisfactory.

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PLATE 1

FIG. 1. A. rutgersensis Waksman and Curtis; old culture on Czapek's agar.

FIG. 2. A. no. 2, an undescribed species; compact, colorless colonies, scant aerial mycelium.

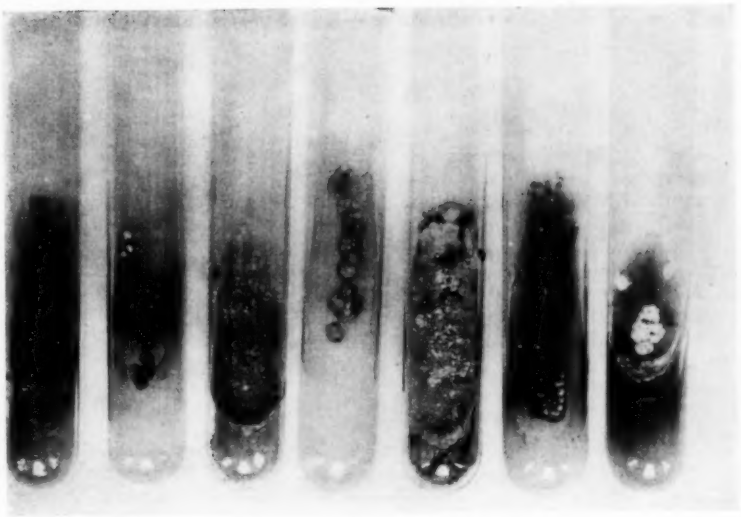
FIG. 3. A. griseus Krainsky, old culture on Czapek's agar.

FIG. 4. A. no. 4, similar in some respects to A. violaceus-caesari, W. and C. Conn's agar, twenty days.

FIG. 5. A. diastaticus Krainsky, twenty-day culture on Czapek's agar.

FIG. 6. A. violaceus-ruber Waksman and Curtis, old culture on Ashby's nitrogen free agar.

FIG. 7. Same as figure 6; twenty-day culture showing aerial mycelium on Czapek's agar.



1 2 3 4 5 6 7

PLATE 2

FIG. 1. A. flavus Krainsky, old culture on Krainsky's calcium malate agar.

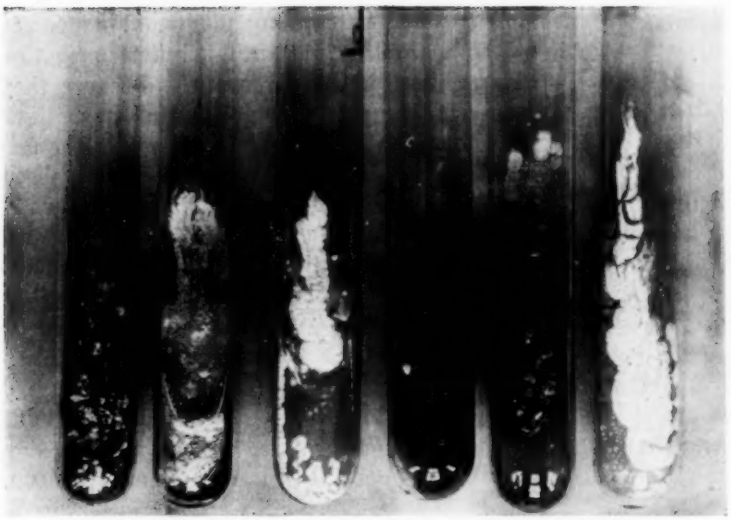
FIG. 2. A. lipmani Waksman and Curtis, showing abundant, spreading growth with gray aerial mycelium on Conn's agar.

FIG. 3. A. albus Krainsky, fifteen-day growth on Conn's agar showing dense aerial mycelium.

FIG. 4. A. boboli Waksman and Curtis, Conn's agar, coral-red colony with no aerial mycelium.

FIG. 5. A culture resembling somewhat A. albosporeus Krainsky, aerial mycelium formed late if at all. Growth on Conn's agar after thirty days.

FIG. 6. A. albus Krainsky, Czapek's agar after fifteen days showing prolific aerial mycelium.



1 2 3 4 5 6

THE CARE OF MORNING MILK BEFORE PASTEURIZATION[1]

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The quality of the raw milk supply is always a matter of concern to the operators of pasteurizing plants. Fortunately those located in the smaller cities are in a much better position to improve it than are those in the larger cities because they are nearer to the source of supply. One of the problems, which is often confronted, is whether or not it is necessary for the farmer to cool his morning's milk before it is brought to the plant for pasteurization. In most of these smaller cities the farmer is located within a few miles of the plant and makes his own deliveries within a few hours after the morning milking. During the summer season he is always in a hurry and if the time and labor involved in cooling the milk could be eliminated he would feel relieved. The actual necessity for cooling this morning milk is often questioned by the producer. The dealer and the health officer are themselves sometimes undecided about it. The frequency with which the advice of the dairy division has been asked on this point has led to the tests here reported.

PURPOSE OF INVESTIGATION

With this problem in mind, a brief study was made to determine the practicability of such a plan. The study did not contemplate a further investigation of the so-called "germicidal" property of fresh milk, nor was any elaborate preparation of utensils or control of other conditions attempted. The purpose of the work was to note what changes might take place in the bacterial count of an average can of milk as it might be found on any farm at the time of milking or during the period

[1] Published with the approval of the director as paper no. 325, Journal Series, Minnesota Agricultural Experiment Station.

previous to delivery at the plant for pasteurization. No effort was made to select any particular milk or cans. They might have represented those from any average plant or farm, no better and no worse. Some cans were clean and dry, others fairly clean but damp. On some days the temperature of the outdoor air was high, on others moderate. The conditions represented ordinary daily variations during the summer months.

METHOD OF PROCEDURE

At the time of the morning milking, a can was chosen at random from those ready for use in the milk room. It was filled at once with fresh milk. A sample of this was plated immediately on standard lactose agar. The plates were incubated at 37° for forty-eight hours. The temperature of the milk was noted and recorded.

The covered can was allowed to remain undisturbed at the temperature of the room during the remainder of the period. Subsequent platings were made at intervals of one hour for five consecutive hours, and the temperatures recorded.

EXPERIMENTAL DATA

The bacterial counts of the samples, arranged according to the initial counts, are given in table 1.

It will be noted that there is a marked variation in the bacterial content of the fresh milk, a few representing milk of excellent quality, while the majority show a remarkably high count. It may be said that the majority of the cans were damp, as might be the case on the average farm. A number of the samples show a decrease in the count during the first two or three hours. They are distributed widely. Of the 24 samples, at the end of one hour, 10 had a lower count than the original; after two hours, 8; and after three hours, only 1.

A bacterial count of 1,000,000 per cubic centimeter by the agar plate method, is accepted in many localities as the maximum for milk to be used for pasteurization.

In looking over the data, it may be noted that 50 per cent of the samples exceed this standard at the end of four hours. Those

TABLE 1

Bacterial count by agar plate method

SAMPLE NUMBER	BACTERIAL COUNT PER CUBIC CENTIMETER					
	Original	After one hour	After two hours	After three hours	After four hours	After five hours
1	1,770	1,780	2,000	2,070	3,100	8,370
2	1,820	880	1,340	3,900	6,700	8,000
3	6,800	1,800	4,400	3,100	12,500	19,600
4	10,900	8,900	2,200	29,600	33,900	42,000
5	12,500	18,800	24,000	35,000	25,000	50,000
6	14,900	17,200	19,300	53,000	88,000	201,000
7	104,000	214,000	290,000	296,000	410,000	900,000
8	109,000	83,000	81,000	191,000	470,000	1,600,000
9	138,000	146,000	102,000	246,000	288,000	570,000
10	161,000	143,000	353,000	375,000	1,600,000	2,340,000
11	176,000	180,000	244,000	325,000	370,000	530,000
12	177,000	320,000	490,000	1,000,000	1,980,000	5,500,000
13	181,000	332,000	380,000	410,000	1,400,000	2,250,000
14	205,000	253,000	298,000	330,000	600,000	770,000
15	219,000	200,000	215,000	480,000	1,760,000	2,860,000
16	224,000	215,000	270,000	800,000	1,590,000	2,800,000
17	241,000	320,000	470,000	1,000,000	2,600,000	4,400,000
18	243,000	275,000	400,000	1,120,000	1,600,000	2,460,000
19	287,000	370,000	430,000	1,510,000	3,300,000	5,500,000
20	370,000	470,000	600,000	1,550,000	4,500,000	10,200,000
21	422,000	230,000	97,000	730,000	780,000	740,000
22	480,000	330,000	270,000	1,670,000	2,880,000	4,500,000
23	760,000	910,000	1,400,000	4,900,000	6,700,000	9,500,000
24	1,390,000	540,000	2,320,000	3,800,000	3,700,000	9,200,000
Average	247,320	232,515	365,135	869,153	1,529,050	2,789,540

with an original count of less than 100,000 per cubic centimeter are still well below this limit even after five hours.

The 24 samples may be divided into groups of 12 on the basis of the original count as follows:

	AVERAGE COUNT	
	Original	Final
Lowest (12).....	76,140	418,500
Highest (12).....	418,500	4,593,166

From this it will be noted that the group showing the lower original count also shows the lower final count.

In a comparison of an average of the 12 samples showing the lowest final count, it will be found that they also show the lower average original count.

Table 2 is a summary of the temperatures observed at the various intervals. The decrease in temperature is most marked during the first hour and very gradual during the remainder of the period, changing but slightly after the third hour.

TABLE 2

Range of temperatures at each interval during the five-hour period

PERIOD	MAXIMUM	MINIMUM	AVERAGE
	°F.	°F.	°F.
Original	96.8	86.0	92.1
After one hour	93.2	72.5	84.5
After two hours	87.8	72.5	82.0
After three hours	86.0	73.4	80.6
After four hours	85.1	72.5	80.0
After five hours	86.0	72.5	79.7

The relation between the decrease in temperature during the five-hour period and the original and final counts is shown in the following:

NUMBER OF SAMPLES	AVERAGE DECREASE IN TEMPERATURE	AVERAGE COUNT	
		Original	Final
	°F.		
12	10.5	143,395	1,663,000
12	14.1	96,381	1,126,544

The temperature throughout the period is such that it would be favorable for very active growth of most species. Consequently the slight difference between the two groups from the standpoint of the decrease in temperature is relatively insignificant. The initial count of the sample appears to be of much greater importance than the temperature.

SUMMARY AND CONCLUSIONS

The results of the study would indicate, as already generally understood, that the change in the bacterial content of milk during the first few hours after milking will depend upon:

1. The so-called "germicidal property" of certain individual samples.
2. *The initial count* (contamination from all sources).
3. The temperature at which it is held.
4. The time elapsing.

If milk is obtained from reasonably clean cows which do not give a high initial count (i.e., normally or because of any inflammation of the udder such as mastitis), milked into clean, *dry* utensils (especially milking machines and cans) and delivered to the pasteurizing plant within five or six hours after milking without cooling, the bacterial content should not be excessive for use for pasteurization. However, under average farm and plant practices, these conditions are not always to be found, particularly the clean, dry utensils. If the utensils are not clean and dry the initial count will tend to be so high that it will be impossible for the dairyman to deliver a milk which is satisfactory for pasteurization without cooling it adequately at once. In communities where the conditions can be satisfactorily controlled the farmer might be permitted to deliver uncooled morning milk to the pasteurizing plant. However, as a general practice in all communities it is not to be recommended.

LOSS IN THE GRAIN OF CORN IN STORAGE AS SILAGE

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The principle of ensiling corn involves two possibilities of loss: (1) Utilization of the silage may necessitate the feeding of more grain than is profitable; (2) the grain itself may suffer a loss of nutrients.

Emphasis is placed on the grain since it constitutes two-thirds the nutritive value of the crop and practically its entire merchantable value. (Reference is made to the present extensive practise of growing and harvesting corn for silage in such way as to secure a maximum yield of sound grain.) Nevertheless, corn is harvested as silage not as a better method of harvesting the grain or to enhance its feeding value, but purely to better utilize the stalk for feeding purposes. The continued and increasing use of corn silage is, of course, the best of evidence that the superior two-thirds of the crop represented by the grain is not seriously injured simply to make better use of the inferior one-third represented by the stalk.

At the time of harvest the grain has a definite cash value. Immediately upon harvest this value is destroyed since it is no longer merchantable. It still retains a feeding value but must be fed in such amount as is present in the silage ration. An ordinary silage ration of 35 pounds contains the equivalent of 5 pounds of dry shelled corn. It may be unprofitable to feed corn in such amount depending on the price of corn and the feeding use made of it. When corn was selling above a dollar a bushel the point became acute and a continuation of the high price level would have undoubtedly greatly curtailed the use of the crop for silage. So far as the dairyman is concerned he will usually want to feed corn in as large amount as that present in the silage ration and so is not particularly concerned with this feature of possible loss.

There remains the second possibility, namely, loss of nutritive value in storage. The present paper gives a limited amount of data bearing on this point but is offered more particularly as giving a method of attacking the problem. The method is based on the fact that in the mechanical preparation of the crop the kernels are practically all shelled from the cob and the majority pass into the silo intact. Some are cut and crushed but those which escape uninjured should be representative of the whole. By taking the kernel as a unit and using a number sufficiently large it is possible to determine the changes occurring in the grain under perfectly normal conditions of storage. The number of kernels to be used will be determined by the variability of the kernels themselves in respect to the qualities under study.

In a preliminary way, the dry matter changes in the grain as stored in silo and crib have been determined on the basis of the above method. A wood stave silo was filled September 28 and 29, 1920, from a field of Silvermine corn. Representative rows of the field were left standing and the corn husked and cribbed later. The yield of grain, estimated from these rows was 55 bushels per acre. The yield of silage was about 9 tons per acre. At the time of filling two samples of grain were collected from the silo and the positions marked by boards placed in the silage. When the silage was fed down to the board markers, June 25, and August 10, 1921, samples of grain were again collected. From the grain samples, lots of 100 kernels, intact but otherwise selected at random were used as subsamples. The cribbed corn from the rows left standing in the field was shelled March 16, 1921, and sampled. Lots of 300 kernels selected at random were taken as subsamples. Moisture was determined by the Brown-Duvel method.[1] Results are given in the table.

The table shows the kernel takes up moisture in storage as silage and that the percentage water content is increased from 35.85 as stored to 39.44 after storage. Evidently a physical

[1] Circular no. 72, Bureau of Plant Industry, U. S. Department of Agriculture. The method was modified by holding the temperature at 110°–115° for ten minutes with the high moisture samples in order to prevent excessive oil being carried over by the rapid evolution of steam.

balance is established with reference to water between the kernel and its surrounding medium. Moisture content of the silage was not determined but was probably around 70 per cent. Kernels saturated with water by soaking thirty hours contained 46.35 per cent of water.

TABLE 1

Loss of dry matter in the grain of corn as harvested and stored in crib and silo

	NUMBER OF KERNELS USED	AVERAGE PER KERNEL				LOSS OF DRY MATTER
		Weight	Dry matter	Water	Dry matter	
		grams	*grams*	*per cent*	*per cent*	*per cent*
As harvested in silo	1900	0.4573	0.2934	35.85	64.15	
After storage in silo	1500	0.4599	0.2785	39.44	60.56	5.08
After storage in crib	1200	0.3382	0.2868	15.20	84.80	2.25

The per cent of dry matter in the grain is decreased by the absorption of water, but there is also an actual loss of dry matter. Based on the dry matter present at time of harvest this loss amounts to 5.08 per cent. The cribbed corn also showed a loss of dry matter amounting to 2.25 per cent on the same basis. The loss in the silo appears to be greater than in the crib but both are small.

Apparent sources of error, outside of variability in the kernels, are: Changes in the crushed kernels may not be the same as in the intact kernels, and there may be a passage of material other than water into or out of the kernel.

SUMMARY

Changes in the grain of corn silage may be determined under normal conditions of storage by using the kernel as a unit and applying statistical methods. Data from one silo show a loss of 5.08 per cent in the dry matter of the grain. The corresponding loss in crib storage was 2.25 per cent.

REVIEW OF FOREIGN DAIRY LITERATURE [1]

GORINI, COSTANTINO, Bacteriological Laboratory, Royal Superior School of Agriculture, Milan, Italy. Physiological Mutations of Bacteria by Means of Individual Divergencies.

The question of mutations is of the greatest importance to the geneticist. It is known that DeVrie's theory is in antithesis to the Darwinian doctrine insofar as it contends that the origin of species does not take place gradually, by adaptation and selection, but abruptly as a result of internal causes. Thus, to the Darwinian concept of continuous variability is to be replaced the concept of discontinuous variation. As a matter of fact there are arguments in favor of both these theories, and it is reasonable to assume that they may coexist.

Controversies have centered upon the definition of the term *Mutation*. The essential characters given by DeVries for this phenonenon are: sudden and spontaneous appearance, lack of finality and the fact that mutations are hereditarily fixed. Each of these characters has been the object of extensive discussion so that now amplifications and limitations have been applied in various cases. Leaving aside the botanical and zoological phases of the subject and viewing the matter solely from the bacteriological standpoint, it should be stated that we are here concerned with delicate and long series of observations which may well lead to errors and erroneous interpretations.

Neisser (1) and Massini (2) were the first authors to mention mutation among bacteria and they were soon followed by a host of imitators; it should nevertheless be mentioned that not all the workers have given sufficient proof of the purity of the cultures they were working with and of the fact, that the variations they observed were not due to external factors, ceasing with the return of these outside conditions.

In fact it is logical to overlook the spontaneity in appearance of the variation under consideration, and to consider as mutations also modifications of experimental origin provided their permanence can be proved after the removal of the causal experimental conditions. One eloquent example in this connection is furnished by the work of Schierbeck (3) who, working with lactic bacteria observed phenomena of attenuation which were constant even in continued transfers from

[1] This article was translated and presented for publication by Augusto Bonazzi, Wooster, Ohio.

milk to milk; so already in 1900, i.e., when mutations were not even spoken of as yet this author assumed he was working with "*lasting varieties.*" Nevertheless when these cultures were transferred into special milk, very old and repeatedly sterilized, Schierbeck found them to regain their original zymogenic powers. He thereby concluded that ordinary milk had an inhibiting effect upon the lactic ferment and that the absence of this inhibiting factor in the special milk allowed these organisms to regain their original vigor. This is a typical example of such pseudomutations as very easily mislead the investigator who does not take into careful consideration such essential precautions as are dictated by the knowledge of the extreme sensitivity of bacteria towards even the slightest variations in experimental conditions.

Principal requisites of these investigations, says DeVries, are accuracy and perseverance.

In order to avoid the nearly inevitable errors in technique which easily creep in during these investigations, familiarity with the bacterial species under examination is necessary; a familiarity which can only be acquired after long periods of cultivation. I have transferred, as an example, my lacto-proteolytic organisms from time to time, and at the variable intervals indicated in my previous communications (4), for the last fifteen to twenty years.

It is by this means that I have been able to detect in every detail even slight differences in behavior towards temperature, aerobiosis, quality of nutritive media (especially milk), differences which lead at irregular intervals to irregular oscillations of the complex acido-coagulating and peptonizing activity. This irregularity I have for the past always considered of a transitory nature, and it is only recently that the repeated appearance of certain phenomena (5) which I had up to the present considered due to contamination or to inevitable accidents in the *modus operandi*, led me to ascertain true mutations characterized by the classical sudden appearance, spontaneity and independence from external factors and by their hereditary characters. This last condition is not to be taken in the absolute meaning of "*perennial constancy;*" it is sufficient that the new character be transmissible for a few generations. I have in fact observed also cases of reversibility, true *retro-mutations*, sudden and transmissible which DeVries himself partially admits.

I have been able to determine a new fact: that we are not dealing here with a transformation involving the whole of a bacterial culture, since not all the parallel transfers from the same mother culture yield the

modification but only some among them. This I have found to be the case although I took the precuation that the mother culture originate from a single cell obtained by the method of Burri.

In an attempt to find an explanation for the above mentioned phenomena I found it necessary to admit the possibility of an individual divergence in the cells of the same species of lacto-proteolytic ferments. An individuality which allowed the classification of the cells into three main types with regard to their saccharolytic and proteolytic properties; one type with these two properties in equilibrium, one other prevalently saccharolytic, the third preeminently proteolytic. According to this interpretation, mutations lose the appearance of abnormality and fall within the realm of normal phenomena subordinated only to the laws of chance (even though rare) whereby the material in the transferred inoculum is made up exclusively or almost exclusively of cells of the same type and falling only within one of the above groups. So that among the many transfers from the same mother culture there may exist side by side with the *mutant* cultures of the original type. In its turn again, the mutant may again at a given moment, and due to the play of chance, give rise on further transfer to a culture typical of the original variety (*retromutation*). Due to chance, is here again emphasized, under conditions of eugenesis and independently of disgenetic conditions as has been claimed by some authors (6). Therefore the possibility, should be admitted among bacteria of a cellular individuality similar to that which has been demonstrated for yeasts isolated by the single cell method, according to the method of Hansen.

Such considerations led me to take up again the study of several points of dairy bacteriology; to begin with my own personal researches.

In my first contribution upon the subject of the mammary flora (7) I listed five types of cocci which already at that time I would not attempt to consider as separate species but only as physiological types, since I found them to be distinctly linked by even ever-so-distant similarities.

In my other works upon the subject, I did not emphasize these distinctions but limited myself to the qualification of the mass of mammary cocci as lacto-proteolytic, since I was then convinced of the difficulties entailing a sharp differentiation of these organisms into peptonizing and non-peptonizing cocci. In fact I had been using as a criterion of the diversity of these five types their behavior in gelatine and milk, since I had at first noticed a certain similarity between the peptonizing powers on gelatine and that same power on casein. Nevertheless already at that time my attention had been drawn to the appearance

here and there of such types as I designated in my notes of the time, "*mixed types*" and which were to be classified in different groups according to whether they were considered with regard to their behavior in milk or gelatine.

Further studies (8) led me to call attention to the existence of cocci incapable of dissolving gelatine, but capable instead of dissolving casein, and still later I noticed the contrary phenomenon of gelatine-liquefying cocci which when placed in milk would coagulate but not peptonize the casein over long series of transfers. But now, benefiting by my later experience on the phenomena of mutation, I am in a position to state that if many subcultures are prepared from the same mother culture, liquefying or non-liquefying, discordant results may be obtained, i.e., daughter cultures which in some cases have simply coagulating and in other cases both coagulting and peptonizing properties.

A second group of organisms to which this line of reasoning, I thought, would be worth extending is that of the Streptococcus lacticus, an organism which according to my researches (9), now reconfirmed (10), is capable of peptonizing casein only at low temperatures of incubation. Also in this case I found that a single colony can yield transfers which when placed in milk are capable of different proteolytic powers, in some cases failing entirely in this property for some unknown reason and independently of the quality of the milk as well as of the experimental procedures. This together with other reasons pointed to in earlier publications, may explain why various authors failed to ascertain peptonizing properties in Streptococcus lacticus.

Observations of a similar nature I made on a third group of lactoproteolytic bacteria of milk, i.e., Lactobacillus sporificans. This group is typified by the B. acidificians-presamigenes-casei which I described in 1904 (11) in cheese and which I considered as a first example of a spore-forming lactic ferment. Others were added to the list, some isolated from silage (12) and others from fermented milk products (13). A new type has recently been isolated and described in butter by Sandelin (14).

In view of the above considerations it is now more appropriate to classify these three groups into variations closely associated with individual divergencies among cells of the same species rather than peptonizing or non-peptonizing species or races. These variations may be transitory, due to the *modus operandi* or other circumstances favoring either saccharolysis or proteolysis, and again there may be permanent and transmissible acquiring thereby the characters of mutations due to

the element of chance in the make up of the transfer inoculum as has been stated above.

How many of the properties of the lactic ferment are subject to these mutations due to simple individual divergencies rather than to adaptations or forced modifications? The Gram-staining properties may be mentioned in this connection properties which have been by some investigators assumed useful in the differentiation of true from pseudo-lactic ferments. Here again we may mention the classical example of B. coli so closely allied to the lactic bacteria, its fermentative affinities and its innumerable physiological races isolated, which have also been made to include the one of B. coli mutabile. Are these true races or cellular individualities within the same race?

These investigations lead me to caution against the eventual appearance of mutations in the selected cultures such as are used in the dairy. To avoid such mutations it is advisable to add to the scrupulous identity of developmental conditions, the caution of using abundant inoculi taken from the whole depth of the mother culture, since it should be considered possible that the various cellular types occupy different layers, or zones, in the growth.

Nevertheless my long experience has shown that these precautions are not in themselves sufficient, since, in spite of their constant adoption in my transfers, modifications have crept into my cultures for butter and for cheese manufacture. Although up to the present time I had leaned towards the assumption of weakenings or degenerations under unnatural conditions, I am now more inclined to consider these modifications as consequences of cellular individuality since I was able to show that even in these cases normal cultures may be obtained if the precaution be taken of making several parallel transfers.

Naturally, although mutations are not caused by environmental conditions, they are apt to be more sharply differentiated from the original type under unfavorable conditions. It is therefore to be recommended in making "*selected cultures*" to use media that will further a rapid development and to make several parallel transfers.

These phenomena of cellular individuality may be used in an attempt to overcome the difficultues attending the classification of the lactic bacteria even though the physiological criteria be used in preference to the morphological according to my findings (4).

A clear example of this is to be found in the intricate systematization, recently proposed by Orla Jensen, and based on cultural-biochemical complexes (15). It must be admitted that the attempt is laudable but

also that the results obtained fall far short of expectation and this due to the numerous uncertainties encountered. Taking as an example the criteria based upon the presence or absence of acido-proteolytic properties we find Jensen to confirm my previous work when he finds his property present in several of his lactic ferments, but I will also state that, had he not submitted his milk to the drastic sterilization which he recommends in his Treatise (16) he would have found this property far more widespread conforming to my findings purporting to show (17) that excessive sterilization of milk renders this substance unfit for the demonstration of caseolytic properties of the lactic bacteria. Now, Jensen having observed the presence of this property in some forms and its absence in others, makes of it a criterion for the differentiation of species.

From the standpoint of the action of these organisms on the carbohydrates, Jensen distinguishes species fermenting some sugars from others which never do so, as well as those which may or may not attack them according to circumstances. In an attempt to explain these inconsistencies he recognizes in the species, tendencies to undergo weakenings or degenerations. It is only natural if we advance the following question: Why do not the other species show these tendencies? If we admit the influence of cellular individualities, we find a plausible explanation for these phenomena, not only, but actually avoid the danger of creating new species and a new terminology not based on sufficiently sound criteria.

SUMMARY

In previous contributions I have shown that the group of the acido-proteolytic lactic ferments presents a great variability and irregularity in the manifestation of their coagulating and peptonizing properties according to external conditions as well as the *modus operandi* in experimental procedure.

In a recent note I have shown that they are capable of showing phenomena of true mutation, spontaneous, sudden and transmissible: having, nevertheless, found these mutations localized in portions of a culture I have recognized in them normal variations in cellular individuality.

In the present contributions I attempt to show that these considerations throw a new light upon a number of questions interesting dairy bacteriology: questions relating to the qualitative and quantitative irregularities to be observed in the activities of these organisms, dis-

tinguishable from errors of technique and observation, responsible for the endless and ever insufficient number of species, subspecies, races and varieties, created by investigators, as well as for the process of hypothetical attenuation or degeneration which do not find adequate justification in disgenetic conditions. These individual divergencies are also responsible for the ever increasing tendency towards the acceptance of the terminology *group* and *type* in place of *species*, contrary to the dignity of the science of bacteriology.

On the principle of normal cellular individuality we are in position not only to dispense with the necessity of explaining mutations and the conditions leading thereto, but also to simplify the general classification of the lactic ferments, contrary to the modern tendency inclining towards excessive complication.

These researches also find practical application in the culture, and selection of cultures, of lactic bacteria in the laboratory and the industry. In fact although the so-called mutations are inherent to the cell itself, not dependent upon external conditions or the modus operandi, we can easily see how their appearance will be propitiated by the use of small inoculi and by such conditions of culture as tend towards the enhancement of these "*normal*" cell divergencies. Therefore we must recognize the advisability of using large inoculi of taking these inoculi from all portions of the mother culture, and of making several parallel transfers and of using appropriate media such as milk which, according to my teachings, must be absolutely fresh and not drastically sterilized (tyndalized rather than autoclaved).

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TENNESSEE UNIVERSITY

Prof. C. E. Wylie of the Dairy Department reports the following news: Mr. Thomas B. Harrison, a graduate of Purdue University, has accepted the position of instructor in dairying at the University of Tennessee. Mr. Harrison will hold the position formerly held by Mr. Myron A. Loomis, now manager of the Jersey Farm and Milk Company, Nashville, Tenn.

The Dairy Department of the University of Tennessee is conveniently located on the first floor of the new $265,000 Agricultural Building. Recently $11,000 worth of new equipment has been installed for the activities of the department. All equipment in the creamery is new and modern and is operated by individual electric motors for each piece of machinery. The equipment includes a 5-ton refrigerator outfit, two 200-gallon pasteurizing vats, one 600-pound churn, scales, sterilizers, bottles, cans, etc. The creamery is operated on the commercial basis, buying cream for the manufacture of creamery butter, and buying milk for pasteurization and bottling for city supply.

CALIFORNIA UNIVERSITY

Prof. C. L. Roadhouse reports that the new Dairy Industry Building at Davis is nearly completed and will be ready for use at the opening of the school year in August.

MASSACHUSETTS AGRICULTURAL COLLEGE

Prof. W. P. B. Lockwood resigned as head of the Dairy Department, effective April 1. He has accepted a position as managing director of the New England Dairy and Food Council, with headquarters at 51 Cornhill, Boston, Mass. Professor Lockwood will, however, continue to spend about one-third of his time with the college as extension professor in dairying. Prof. H. F. Judkins has been appointed acting head of the dairy department.

J. H. F.

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